WATER ANALYSIS HANDBOOK

7th Edition

Photometric Procedures
Titration Procedures
Ion-Selective Electrode Procedures
Microbiological Procedures
Chemical Procedures Explained
Digesting Liquids, Oils and Solids

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Applications Guide

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Acid/Base										•			•	•				•	•						
Acidity		•				•			•	•	•		•					•	•		•				•
Alkalinity	•	•	•	•	•	•			•	•	•	•			•	•					•	•		•	•
Aluminum			•		•	•			•									•	•	•			•	•	
Arsenic									•	•	•						•			•			•	•	•
Ascorbic Acid				•																					
Bacteria		•		•	•				•	•	•	•		•	•	•	•	•	•	•		•	•	•	•
Barium						•					•			•	•								•		
BOD				•					•	•	•	•		•	•			•		•	•		•	•	
Boron					•	•				•	•			•	•		•					•	•		•
Bromine				•	•				•		•					•						•		•	
Cadmium						•			•				•						•	•			•	•	
Calcium	•			•	•						•				•							•			•
Carbon Dioxide		•		•	•				•	•	•														•
Chelants					•	•			•								•								•
Chloride	•	•		•	•	•	•		•	•	•	•	•	•	•		•		•		•	•	•	•	•
Chlorine	•	•	•	•	•		•	•	•	•	•	•		•	•	•	•	•			•	•	•	•	•
Chlorine Dioxide				•	•				•			•				•		•			•				
Chromate													•						•						
Chromium (Hexavalent)					•	•			•	•	•		•	•									•	•	
Chromium (Total)					•	•				•	•		•	•						•	•		•	•	•
Cobalt						•				•	•		•	•									•	•	
COD				•		•	•	•	•	•	•	•		•	•		•	•		•	•		•	•	
Color				•					•	•	•				•			•			•		•	•	
Conductivity	•	•		•	•	•			•	•	•			•			•					•	•	•	
Copper		•	•	•	•	•			•	•	•		•			•	•		•	•	•	•	•	•	•
Cyanide									•		•		•		•								•	•	
Cyanuric Acid						•			•		•					•									
Detergents						•				•	•													•	•

	Agriculture	Aquaculture	Aquarium Testing	Beverages/Bottled Water	Boiler/Cooling Water	Chemical Manufacture	Chlorine Production	Commercial Laundries	Drinking Water	Education	Environmental Testing	Food/Feed Industry	Metals/Mining, Mfg & Finishing	Petroleum Industry	Pharmaceutical Manufacture	Pools & Spas	Power Plant Utilities	Pulp & Paper Mills	Semiconductor Manufacture	Solid Waste/Sludge	Textile Industry	Ultrapure Water	Wastewater, Industrial	Wastewater, Municipal	Water Conditioning
Dissolved Oxygen	•	•		•	•			•	•	•	•	•						•			•	•	•	•	•
Erythorbic Acid					•	•								•			•								•
Fluoride				•					•										•			•	•		•
Formaldehyde						•					•										•				•
Gluteraldehyde						•			•	•														•	
Glycols						•			•		•			•			•								•
Hardness	•	•	•	•	•	•		•	•	•	•	•		•	•	•	•	•			•	•	•	•	•
Hydrazine					•												•								
Hydrogen Peroxide						•			•	•	•		•		•				•			•			•
Hydrogen Sulfide						•			•					•										•	•
Iodide						•																			
Iodine			•	•		•			•		•	•			•									•	
Iron (Ferrous)					•	•			•				•												•
Iron (Total)	•		•	•	•	•		•	•	•	•	•		•			•				•	•	•	•	•
Lead			•						•	•	•		•				•		•	•			•	•	•
Manganese				•			•		•														•		•
Mercury							•		•											•			•	•	•
Molybdenum				•	•	•	•				•				•		•			•	•		•		•
Nickel						•			•		•		•							•			•	•	•
Nitrogen Ammonia	•	•	•		•	•			•	•	•	•		•			•				•	•		•	•
Nitrogen (Inorganic)						•				•	•													•	
Nitrogen (Total)						•			•	•	•										•		•	•	
Nitrogen (Nitrate)	•	•	•	•	•				•	•	•	•									•	•	•	•	•
Nitrogen (Monochloramine)									•														•	•	
Nitrogen (TKN)	•					•			•	•	•	•								•			•	•	
Nitrogen (Nitrite)		•	•		•				•	•	•													•	
Oil and Grease						•						•	•	•						•			•		
Oxygen Scavenger					•	•								•			•								•

	Agriculture	Aquaculture	Aquarium Testing	Beverages/Bottled Water	Boiler/Cooling Water	Chemical Manufacture	Chlorine Production	Commercial Laundries	Drinking Water	Education	Environmental Testing	Food/Feed Industry	Metals/Mining, Mfg & Finishing	Petroleum Industry	Pharmaceutical Manufacture	Pools & Spas	Power Plant Utilities	Pulp & Paper Mills	Semiconductor Manufacture	Solid Waste/Sludge	Textile Industry	Ultrapure Water	Wastewater, Industrial	Wastewater, Municipal	Water Conditioning
Ozone		•		•	•			•	•		•				•							•		•	•
PCB											•						•			•		•	•	•	
Permanganate									•								•								
pH	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Phenols						•				•	•			•			•				•		•		•
Phosphate	•	•	•	•	•	•	•		•	•		•					•	•		•	•	•	•	•	•
Phosphonates					•	•			•	•	•													•	•
Phosphorus	•					•	•		•	•	•	•		•			•				•		•	•	•
Potassium	•								•		•			•								•			
QAC					•	•					•							•							•
Salinity	•	•				•			•	•	•			•						•					•
Selenium									•	•	•				•					•			•		•
Silica	•			•	•	•	•		•	•	•	•			•		•	•	•		•	•			•
Silver						•			•	•	•		•	•	•	•	•	•	•	•			•	•	•
Sodium	•			•					•								•	•	•			•	•	•	•
Sodium Chromate									•	•	•						•								
Sodium Hydroxide													•						•						
Sulfate	•					•			•	•	•						•				•	•	•		•
Sulfide						•			•		•			•									•	•	•
Sulfite					•	•			•	•	•						•	•					•	•	•
Tannin					•				•		•														•
TDS		•		•	•				•	•													•		•
Toxicity						•			•		•									•			•	•	
TPH						•			•		•						•						•		
Triazole					•	•											•								
Turbidity		•		•	•				•	•	•	•	•		•		•	•	•	•		•	•	•	•
Volatile Acids									•	•	•			•										•	
Water in Oil											•		•	•											
Zinc					•	•			•	•	•		•					•		•	•		•	•	•

Section 1 Abbreviations and Conversions

1.1 Procedure abbreviations

The abbreviations in *Abbreviations* table are common in written chemical procedures:

Table 1 Abbreviations

Abbreviation	Definition	Abbreviation	Definition
°C	degree(s) Celsius (Centigrade)	**L**	liter—volume equal to one cubic decimeter (dm^3)
°F	degree(s) Fahrenheit	**LR**	low range
ACS	American Chemical Society reagent grade purity	**MDL**	method detection limit
APHA Standard Methods	*Standard Methods for the Examination of Water and Wastewater,* published jointly by the American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Environment Federation (WEF), is the standard reference work for water analysis. Order from Hach Company, requesting Catalog No. 2270800 or from the Publication Office of the APHA. Many procedures contained in this manual are based on *Standard Methods.*	**MDB**	marked dropping bottle
		mg/L	milligrams per liter (ppm)
		µg/L	micrograms per liter (ppb)
		mL	milliliter—1/1000 of a liter. It is approximately the same as a cubic centimeter (and is sometimes called a “cc”).
		MR	medium range
		NIPDWR	National Interim Primary Drinking Water Regulations
AV	AccuVac®	**NPDES**	National Pollutant Discharge Elimination System
Bicn	bicinchoninate	**P**	phosphorus
conc	concentrated	**PCB**	poly chlorinated biphenyl
DB	dropping bottle	**ppb**	parts per billion
DBP	disinfection by-products	**ppm**	parts per million
CFR	Code of Federal Regulations	**RL**	Rapid Liquid™
EDL	Estimated detection limit	**SCDB**	self-contained dropping bottle
EPA	Environmental Protection Agency	**THM**	trihalomethane
F&T	free and total	**TNT**	Test ‘N Tube™
FM	FerroMo®	**TOC**	total organic carbon
FV	FerroVer®	**TPH**	total petroleum hydrocarbons
FZ	FerroZine®	**TPTZ**	2,4,6-Tri-(2-Pyridyl)-1,3,5-Triazine
g	grams	**USEPA**	United States Environmental Protection Agency
gr/gal	grains per gallon (1 gr/gal = 17.12 mg/L)	**ULR**	ultra low range
HR	high range		

1.2 Conversions

1.2.1 Chemical species

Species conversion factors for many commonly used chemicals are listed in the *Conversion factors* table.

Table 2 Conversion factors

To Convert From...	To...	Multiply By...
mg/L Al	mg/L Al_2O_3	1.8895
mg/L B	mg/L H_3BO_3	5.7
mg/L Ca-$CaCO_3$	mg/L Ca^{2+}	0.4004
mg/L $CaCO_3$	mg/L Ca^{2+}	0.4004
mg/L $CaCO_3$	mg/L Mg^{2+}	0.2428
µg/L Carbo.	µg/L Hydro.	1.92
µg/L Carbo.	µg/L ISA	2.69
µg/L Carbo.	µg/L MEKO	3.15
mg/L Cr^{6+}	mg/L CrO_4^{2-}	2.231
mg/L Cr^{6+}	mg/L Na_2CrO_4	3.115
mg/L Cr^{6+}	mg/L $Cr_2O_7^{2-}$	2.077
mg/L Mg–$CaCO_3$	mg/L Mg^{2+}	0.2428
mg/L Mn	mg/L $KMnO_4$	2.876
mg/L Mn	mg/L MnO_4^-	2.165
mg/L Mo^{6+}	mg/L MoO_4^{2-}	1.667
mg/L Mo^{6+}	mg/L Na_2MoO_4	2.146
mg/L N	mg/L NH_3	1.216
mg/L N	mg/L NO_3^-	4.427
mg/L Cl_2	mg/L NH_2Cl	0.726
mg/L Cl_2	mg/L N	0.197
mg/L NH_3–N	mg/L NH_3	1.216
mg/L NH_3–N	mg/L NH_4^+	1.288
mg/L NO_2^-	mg/L $NaNO_2$	1.5
mg/L NO_2^-	mg/L NO_2^-–N	0.3045
mg/L NO_2^-–N	mg/L $NaNO_2$	4.926
µg/L NO_2^-–N	µg/L $NaNO_2$	4.926
mg/L NO_2^-–N	mg/L NO_2^-	3.284
µg/L NO_2^-–N	µg/L NO_2^-	3.284
mg/L NO_3^-–N	mg/L NO_3^-	4.427
mg/L PO_4^{3-}	mg/L P	0.3261
µg/L PO_4^{3-}	µg/L P	0.3261
mg/L PO_4^{3-}	mg/L P_2O_5	0.7473
µg/L PO_4^{3-}	µg/L P_2O_5	0.7473
mg/L SiO_2	mg/L Si	0.4674
µg/L SiO_2	µg/L Si	0.4674

1.2.2 Hardness conversion

See the *Hardness conversion factors* table for the factors to convert hardness from one unit of measure to another. For example, to convert mg/L $CaCO_3$ to German parts/100,000 CaO, multiply the value in mg/L x 0.056.

Table 3 Hardness conversion factors

Units of Measure	mg/L $CaCO_3$	British gr/gal (Imperial) $CaCO_3$	American gr/gal (US) $CaCO_3$	French Parts/ 100,000 $CaCO_3$	German Parts/ 100,000 CaO	meq/L[1]	g/L CaO	lb/cu ft $CaCO_3$
mg/L CaCO3	1.0	0.07	0.058	0.1	0.056	0.02	$5.6x10^{-4}$	$6.23x10^{-5}$
English gr/gal CaCO3	14.3	1.0	0.83	1.43	0.83	0.286	$8.0x10^{-3}$	$8.9x10^{-4}$
US gr/gal CaCO3	17.1	1.2	1.0	1.72	0.96	0.343	$9.66x10^{-3}$	$1.07x10^{-3}$
Fr. p/ 100,000 CaCO3	10.0	0.7	0.58	1.0	0.56	0.2	$5.6x10^{-3}$	$6.23x10^{-4}$
Ger. p/100,000 CaO	17.9	1.25	1.04	1.79	1.0	0.358	$1x10^{-2}$	$1.12x10^{-3}$
meq/L	50.0	3.5	2.9	5.0	2.8	1.0	$2.8x10^{-2}$	$3.11x10^{-2}$
g/L CaO	1790.0	125.0	104.2	179.0	100.0	35.8	1.0	0.112
lb/cu ft CaCO3	16,100.0	1,123.0	935.0	1,610.0	900.0	321.0	9.0	1.0

[1] epm/L or mval/L

Note: $\frac{meq}{L} = N \bullet 1000$

Section 2 Laboratory Practices

2.1 Temperature

Most methods perform accurately when the sample temperature is between 20 and 25 °C (68 to 77 °F). A note in the individual procedure will state any special temperature requirements.

2.2 Mixing

Swirling is recommended when mixing samples in a graduated cylinder or a titration flask. Swirling is the gentlest method of mixing and offers the least chance for atmospheric contamination when testing for carbon dioxide and other gases.

1. Grip the cylinder (or flask) firmly with the tips of the thumb and first two fingers (see the *Swirl a cylinder and invert a sample cell* figure).
2. Hold the cylinder at a 45-degree angle and make a circling motion from the wrist.
3. Move the cylinder in an approximately 12-inch circle, creating enough rotation to complete the mixing in a few turns.

Mixing sample in a square sample cell:

1. Grasp the neck of the cell with the thumb and index finger of one hand. Rest the concave bottom of the cell on the tip of the index finger of the other hand.
2. Mix by rotating the cell quickly one way and then in the reverse direction.

Figure 1 Rotate a sample cell

Inverting allows for thorough mixing in a capped sample cell or a mixing cylinder.

1. Hold the cell or cylinder, in a vertical position with the cap on top.
2. Invert so that the cap is on the bottom. Return the cell to its original position (see *Swirl a cylinder and invert a sample cell* figure.) Repeat as needed.

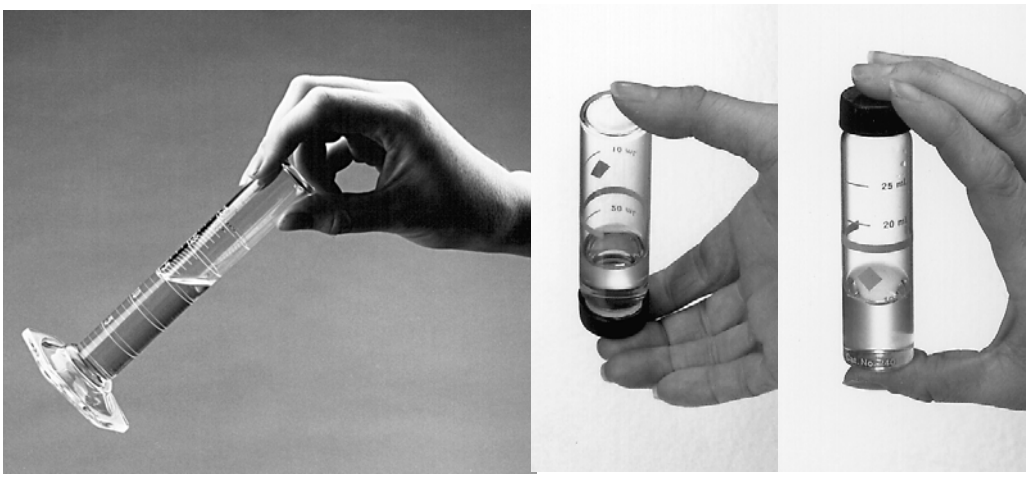

Figure 2 Swirl a cylinder and invert a sample cell

2.3 Digestion

Several procedures require sample digestion. Digestion uses chemicals and heat to break down a substance into components that can be analyzed. This section briefly describes three different digestion procedures.

The Digesdahl system is a process that yields a digest suitable for the determination of metals, total phosphorus and total Kjeldahl nitrogen (TKN). It is rapid, convenient and is very effective at destroying interfering organic materials.

For USEPA reporting purposes, USEPA-approved digestions are required. USEPA presents two digestions (mild and vigorous) for metals analysis. Other digestion procedures are required for mercury, arsenic, phosphorus and TKN.

See *Sample Pretreatment by Digestion* for more information on sample digestion.

2.4 Distillation

Distillation is an effective, easy and safe method of separating some chemical components for analysis. The following equipment is recommended for distillation:

- General Purpose Distillation Apparatus (Catalog No. 2265300), shown in the *General purpose distillation apparatus* figure
- Arsenic Distillation Apparatus Set (Catalog No. 2265400)
- Cyanide Distillation Apparatus Set (Catalog No. 2265800)
- General Purpose Heater and Support Apparatus (Catalog No. 2274400, 115 VAC, 60 Hz)
- General Purpose Heater and Support Apparatus (Catalog No. 2274402, 230 VAC, 50 Hz)

Note: *When ordering the Cyanide or Arsenic Distillation Apparatus, always order in conjunction with the General Purpose Distillation Apparatus and the General Heater and Support Apparatus.*

The Distillation Apparatus is suitable for water and wastewater that requires sample pretreatment by distillation. Applications for the General Purpose Apparatus include: fluoride, albuminoid nitrogen, ammonia nitrogen, phenols, selenium and volatile acids. The General Purpose Heater and Support Apparatus provides efficient heating and anchoring of the glassware.

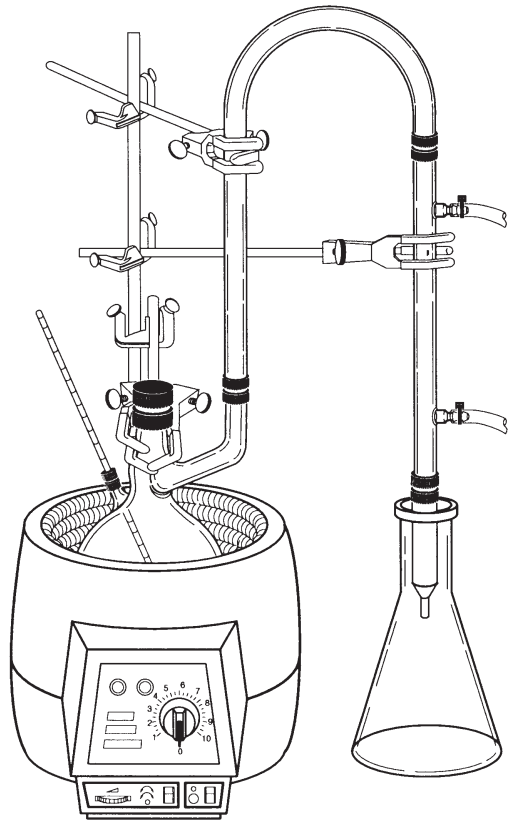

Figure 3 General purpose distillation apparatus

2.5 Filtration

Filtration separates particulates from an aqueous sample. It uses a porous medium that retains particulates but allows liquids to pass through. It is useful for removing turbidity (which may interfere in colorimetric analyses) from water samples.

The two methods most frequently used filtration methods are vacuum and gravity filtration.

2.5.1 Vacuum filtration

Vacuum filtration uses both suction and gravity to draw the liquid through the filter. An aspirator or vacuum pump is used to create suction (see the *Vacuum filtration* figure). Vacuum filtration is faster than gravity filtration alone.

To filter using a vacuum:

1. Use tweezers to place a filter paper into the filter holder.
2. Place the filter holder assembly in the filtering flask. Dampen the filter with deionized water to make sure adhesion to the holder.
3. Position the funnel housing on the filter holder assembly.
4. While applying a vacuum to the filtering flask, transfer the sample to the filtering apparatus.
5. When the filtration is complete, slowly release the vacuum from the filtering flask and transfer the solution from the filter flask to another container.

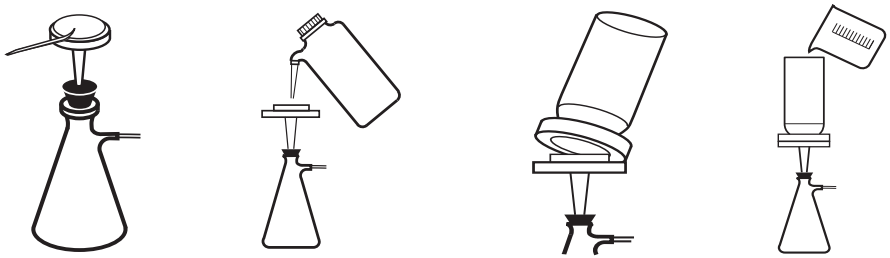

Figure 4 Vacuum filtration

2.5.2 Required apparatus for vacuum filtration

Table 4 Required apparatus for vacuum filtration

Description	Unit	Catalog. No.
Filter Discs, glass fiber, 47-mm	100/pkg	253000
Filter Holder, membrane, 47-mm	each	1352900
Flask, filtering, 500-mL	each	54649
Select one of the following:	—	—
Pump, vacuum, hand operated	each	2824800
Pump, vacuum, portable, 115 VAC	each	1469700
Pump, vacuum, portable, 230 VAC	each	2824801
Tubing, vacuum	—	2074145
Tweezers	each	1428200

2.5.3 Gravity filtration

Many chemical procedures use gravity filtration. This requires filter paper, a conical funnel and a receiving flask (see the *Gravity filtration* figure). Gravity filtration is better for retaining fine particles. The rate of filtration increases as the volume increases in the filter cone, but never fill the cone more than three-quarters full.

Note: *Pretreatment using acid and heat is often necessary for metals testing. Filter paper will not withstand such an environment; therefore, vacuum filtration with glass fiber filter discs is recommended. Also, glass filter discs do not retain colored species like the paper filters do.*

To filter using gravity:

1. Place a folded filter paper into the funnel.
2. Dampen the filter paper with deionized water so it adheres to the funnel.
3. Place the funnel into an Erlenmeyer flask or graduated cylinder.
4. Pour the sample into the funnel.

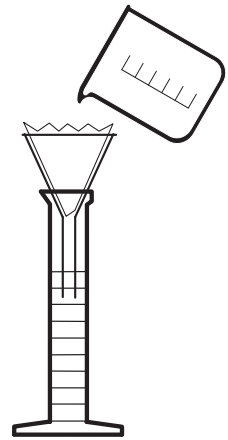

Figure 5 Gravity filtration

Table 5 Required apparatus for gravity filtration

Description	Unit	Catalog. No.
Cylinder, graduated, 100-mL	each	50842
Funnel, poly, 65-mm	each	108367
Filter Paper, 12.5-cm, pleated	100/pkg	189457
Flask, Erlenmeyer, 125-mL	each	50543

2.6 Reagents

2.6.1 Reagent and standard stability

In general, reagents and standards have the maximum shelf life when stored in a location that is cool, dark, and dry. The product label will specify any special storage needs.

It is always good laboratory practice to date chemicals upon receipt and to rotate supplies so the older supplies are used first. When the reagent shelf life is unknown or in doubt, run a standard to check reagent effectiveness.

Absorption of moisture, carbon dioxide or other gases from the atmosphere, bacterial action, high temperatures or light (with photosensitive compounds) may affect the reagent shelf life. In some cases, reaction with the storage container or interaction of reagent components may occur.

2.6.2 Reagent blank

In several tests, the contribution of the reagent(s) to the final reading is of such a magnitude that it must be compensated for whenever the test is performed. **Reagent blank** refers to that portion of the test result contributed solely by the reagent. This produces a positive error in the test results.

Every effort is made to produce reagents with the lowest possible blank; and, for most reagents, it is less than 0.009 absorbance units. However, it is sometimes impossible or impractical to produce reagents with such a low blank. When using such reagents, it is best to determine the reagent blank by performing the procedure using high-quality water (deionized, distilled, etc.) in place of sample to “zero” the instrument. The resulting value is then expressed in the concentration units of the test and is subtracted from each sample determination that uses the same reagent lot. Spectrophotometer software allows the reagent blank value to be stored and subtracted automatically from each sample value. The reagent blank needs to be determined only at first use, when a new lot of reagent has been opened or if contamination is suspected.

In most tests, the reagent blank is so small the instrument may be zeroed on either an untreated portion of the original water sample or on deionized water. This will not result in a significant loss of accuracy unless the test is for very low levels of the species of interest. It is then best to use a reagent blank prepared as above.

2.7 Sample dilution

Most colorimetric tests use volumes of 10 and 25 mL. However, in some tests, the color developed in the sample may be too intense to be measured due to high levels of analyteor or unexpected colors may develop due to an interference. In either case, dilute the sample to produce a measurable endpoint or to determine if interfering substances are present.

To dilute the sample:

1. Pipet the chosen sample portion into a clean graduated cylinder (or volumetric flask for more accurate work).
2. Fill the cylinder (or flask) to the desired volume with deionized water.
3. Mix well. Use the diluted sample when running the test.

The *Sample dilution volumes* table shows relative quantities and the multiplication factors to use with a 25 mL graduated cylinder. The concentration of the sample is equal to the diluted sample result multiplied by the multiplication factor.

Table 6 Sample dilution volumes

Sample volume (mL)	mL Deionized Water used to bring the volume to 25 mL	Multiplication factor
25.0	0.0	1
12.5	12.5	2
10.0[1]	15.0	2.5
5.0[1]	20.0	5
2.5[1]	22.5	10
1.0[1]	24.0	25
0.250[1]	24.75	100

[1] For sample sizes of 10 mL or less, use a pipet to measure the sample into the graduated cylinder or volumetric flask.

More accurate dilutions can be done with a pipet and a 100-mL volumetric flask (See table for *Multiplication factors for diluting to 100 mL*).

1. Pipet the sample and dilute to volume with deionized water.
2. Stopper and invert to mix.stopper and invert to mix.

Table 7 Multiplication factors for diluting to 100 mL

Sample volume (mL)	Multiplication factor
1	100
2	50
5	20
10	10
25	4
50	2

2.7.1 Sample dilution with interfering substances

Sample dilution may influence the level at which a substance interferes. The effect of the interferences decreases as the dilution increases. In other words, higher levels of an interfering substance can be tolerated in the original sample if it is diluted before analysis.

Example:
Copper does not interfere at or below 100 mg/L for a 25 mL sample in a procedure. If the sample volume is diluted with an equal volume of water, what is the level at which copper will not interfere?

$$\frac{\text{Total volume}}{\text{Sample volume}} = \text{Dilution factor}$$

$$\frac{25}{12.5} = 2$$

$$\text{Interference Level} \times \text{Dilution Factor} = \text{Interference level in sample}$$

$$100 \times 2 = 200$$

The level at which copper will not interfere in the diluted sample is at or below 200 mg/L.

2.8 AccuVac® Ampuls

AccuVac Ampuls contain pre-measured powder or liquid vacuum-packed in optical-quality glass ampules.

To use AccuVac Ampuls:

1. Collect the sample in a beaker or other open container.
2. Use one of the following methods to break the tip off the ampule:
   - Use the optional AccuVac Snapper (Catalog. No. 2405200). See *How to use the AccuVac snapper* figure for instructions or;
   - Place the ampule tip well below the sample surface and break the tip off (see *How to use AccuVac Ampuls* figure) against the beaker wall. The break must be far enough below the surface to prevent air from being drawn in as the level of the sample drops.
3. Secure an ampule cap over the tip of the ampule. Invert the ampule several times to dissolve the reagent. The cap protects from broken glass and provides a grip for inserting and removing the ampul from the cell holder. Wipe the ampule with a lint-free cloth to remove fingerprints.

   Note: *Without the cap, the liquid will stay in the ampule when inverted. DO NOT place fingers over broken glass!*

4. Insert the ampule into the sample cell holder and read the results directly.

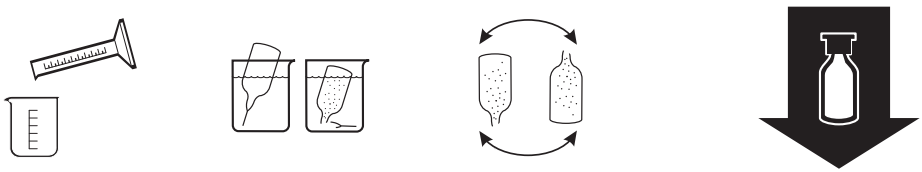

Figure 6 How to use AccuVac Ampuls

2.8.1 Using the AccuVac Snapper

To use the AccuVac Snapper:

1. Hold the Snapper with the open end up.
2. Gently slip the ampule into the Snapper, point first, until the tip touches the ramp at the bottom of the Snapper.
3. Hold the Snapper between the index and middle finger (like a syringe). With the ampule tip down, lower the Snapper into the sample until the ampule shoulder is wet.
4. Press on the flat end of ampule with the thumb (as if depressing the plunger on a syringe) until the tip snaps. Allow the ampule to fill before removing from sample.
5. Rinse the wet end of the Snapper and ampule with clean water, if desired. Remove the ampule from Snapper.
6. Dispose of the ampule tip (retained in the Snapper) in a suitable waste receptacle.

Figure 7 How to use the AccuVac snapper

2.9 PermaChem® pillows

Permachem pillows utilize powdered reagents to minimize deterioration and the risk of reagent spills.

To use PermaChem pillows (see the *How to open PermaChem pillows* figure):

1. Tap the pillow on a hard surface to collect the powdered reagent in the bottom.
2. Locate the tear notch and tear (or cut) across, holding the pillow away from the face.
3. Using two hands, push both sides toward each other to form a spout.
4. Pour the pillow contents into the sample cell and continue the procedure according to the instructions.

Figure 8 How to open PermaChem pillows

2.10 Sample cells

A set of sample cells are shipped with each photometric instrument. The same solution in both cells will give the same absorbance (within ± 0.002 Abs for properly matched cells). For more information, see the section on *Match sample cells*.

For accurate results, use only the sample cells specified in each procedure. Due to differences in cell pathlengths, sample cell substitution will introduce bias in test results. For example, 1-inch square cells have a pathlength approximately 8% longer than 1-inch round cells; substitution of round cells for square cells will introduce a bias in the reading.

2.10.1 Orientation of sample cells

To minimize measurement variability when using a particular cell, always orient the cell in the same manner before placing it into the cell holder. The fill marks on the cells can be used as orientation guides for positioning the cells.

2.10.2 Maintain sample cells

When not in use, store the sample cells in the supplied boxes to protect from scratches and breakage. After use, empty and clean the cells; avoid leaving colored solutions in the cells for extended periods of time.

2.10.3 Clean sample cells

Most laboratory detergents can be used at recommended concentrations. Neutral detergents, such as Liquinox, are safer when regular cleaning is required. Cleaning times are reduced by increasing the temperature or by using an ultrasonic bath. Finish by rinsing a few times with deionized water and allow to air dry.

Cells may also be cleaned with acid, followed by rinsing thoroughly with deionized water.

Note: *Always use acid to clean cells used for low-level metal tests.*

Individual procedures may require special cleaning methods. When using a brush to clean cells, take extra care to avoid scratching the inner surfaces of the cells.

2.10.4 Match sample cells

Although sample cells shipped with the spectrophotometer instrument are distortion-free, nicks and scratches from handling may cause an optical mismatch between two sample cells and introduce error into the test results. This type of error may be avoided by optically matching the sample cells as follows:

Note: *Refer to the spectrophotometer user manual for specific steps required to select wavelengths and zero the instrument.*

1. Power on the instrument. Make sure Display Lock is off or the Reading mode is set to Continuous.
2. Select a wavelength of 510 nm or the wavelength to be used for the test.
3. Pour at least 10 mL (25 mL for 25-mL cells) of deionized water into each of two sample cells.
4. Place one sample cell into the cell holder with the fill mark facing the user.
5. Zero the instrument.
6. Place the other sample cell into the cell holder with the fill line facing the user.
7. Wait for the value to stabilize and read the absorbance. Record the resulting absorbance.

8. Turn the cell 180° and repeat step **6**. Try to achieve an absorbance value within ± 0.002 Abs of the first cell. Note the orientation of the cell.

If the sample cells cannot be matched within ± 0.002 Abs, they may still be used by compensating for the difference. For example, if the second cell reads 0.003 absorbance units higher than the first cell, correct future readings (when using these two cells) by subtracting 0.003 absorbance units (or the equivalent concentration) from the reading. Likewise, if the second cell reads –0.003 absorbance units, add 0.003 absorbance units to the reading.

2.11 Other apparatus

2.11.1 Boiling aids

Boiling is required in some procedures. Under some conditions *bumping* may occur causing sample loss or injury. Bumping is caused by the sudden, almost explosive, conversion of water to steam as it is heated. Use of a boiling aid, such as Boiling Chips (Catalog. No. 1483531), reduces bumping.

Make sure the boiling aids will not contaminate the sample. Do not use boiling aids (except glass beads, Catalog. No. 259600) more than once. Use a large enough flask or beaker to allow significant headspace above the solution. Loosely covering the sample during boiling will prevent splashing, reduce the chances of contamination and minimize sample loss.

Individual procedures will recommend the specific boiling aid to use.

2.12 Achieve accuracy in measurement

2.12.1 Pipets and graduated cylinders

When smaller sample quantities are used, the accuracy of measurements becomes increasingly important. The *Reading the meniscus* figure illustrates the proper way to read the sample level using the meniscus formed when the liquid wets the graduated cylinder or pipet walls.

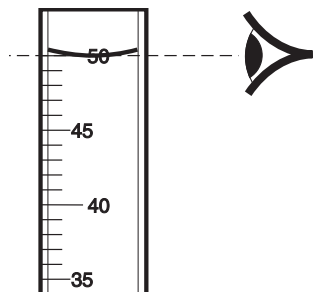

Figure 9 Reading the meniscus

Before filling, rinse the pipet or cylinder two or three times with the sample to be tested. Use a pipet filler or pipet bulb to draw the sample into the pipet.

CAUTION
Never pipet chemical reagent solutions or samples by mouth.

When filling a pipet, keep the tip of the pipet below the surface of the sample as the sample is drawn into the pipet.

Serological pipets have marks that indicate the volume of liquid delivered by the pipet. The marks may extend to the tip of the pipet or may be only on the straight portion of the tube. If the marks are only on the straight part of the tube, fill serological pipets to the zero mark and discharge the sample by draining the sample until the meniscus is level with the desired mark. If the serological pipet has marks extending to the tip of the pipet, fill the pipet to the desired volume and drain all the sample from the pipet. Then use a pipet filler to blow the sample out of the pipet tip with the pipet filler for accurate measurements.

Volumetric (transfer) pipets have a bulb in the middle and a single ring above the bulb to indicate the volume of liquid when it is filled to the mark. To discharge a volumetric pipet, hold the tip of the pipet at a slight angle against the container wall and drain. **Do not** discharge the solution remaining in the tip of the pipet after draining. Volumetric pipets are designed to retain a small amount of sample in the pipet tip.

If droplets of sample cling to the walls of the pipet, the pipet is dirty and is not delivering the correct amount of sample. Wash the pipet thoroughly with a laboratory detergent or cleaning solution and rinse several times with deionized water.

2.12.2 The Pour-Thru™ cell

The Pour-Thru Cell is an optional accessory that improves accuracy and makes measuring more convenient for rapid liquid methods. Methods that use 25-mL samples and sample cells can use the Pour-Thru Cell if specified in the procedure. The Pour-Thru Cell **cannot** be used with 10-mL sample sizes and reagents.*The Cell cannot be used directly with a method unless it is specified in the procedure. For more information, see the photometer user manual.*

See the photometer user manual for installation and operation instructions.

- Pour the solution into the funnel of the installed Pour-Thru Cell Module. Avoid spilling solution onto the instrument.
- The funnel height and orientation may be adjusted. The funnel height determines the speed of sample flow through the cell. The higher the funnel, the faster the flow.
- To minimize air bubbles, adjust the funnel so that it drains completely with the final level of liquid in the tube about 5 cm (2 inches) below the tip of the funnel.
- Take instrument readings after the solution has stopped flowing through the cell.
- Always rinse the cell thoroughly with deionized water after each series of tests or as often as specified in the procedure.

Occasionally, remove the Pour-Thru Cell to check for any accumulation of film on the windows. If the windows appear hazy, soak the cell in a detergent bath and rinse thoroughly with deionized water.

Section 3 Chemical Analysis

3.1 Sample collection, preservation and storage

Correct sampling and storage are critical for accurate testing. Sampling devices and containers must be thoroughly clean to prevent carryover from previous samples. Preserve the sample according to test-specific information about sample preservation.

3.1.1 Collecting water samples

Use a clean container. Rinse the container several times with the water to be sampled before taking the sample. Document the location and procedure used for each sample taken. For example:

From a tap—Take samples as close as possible to the source of the supply. This decreases the influence of the distribution system on the sample. Let the water run long enough to flush the system. Fill sample containers slowly with a gentle stream to avoid turbulence and air bubbles.

From a well—Let the pump run long enough to draw fresh groundwater into the system. Collect a sample from a tap near the well.

From open waters—Sample as near the middle of the body of water as is practical; at least several feet from the shore or edge of the tank. Take the sample under the surface of the water. When using a capped container, submerge it before removin

3.1.1.1 Types of containers

- The *Example* on page 23 lists recommended containers for specific parameters.
- Polypropylene and Polyethylene
- Quartz or TFE (tetrafluoroethylene, Teflon®)— higher quality and price
- Glass—Glass provides a good general-purpose container. Avoid using soft-glass containers to collect samples to be tested for metals in the microgram-per-liter range.
- When determining silver, store samples in dark containers such as amber or brown glass.

Acid washing will thoroughly clean sample containers before use.

3.1.1.2 Acid washing

If a procedure suggests acid washing, follow these steps:

1. Clean the glassware or plasticware with laboratory detergent. Phosphate-free detergent is best. (When determining phosphates, phosphate-free detergent **must** be used.)
2. Rinse well with tap water.
3. Rinse with a 1:1 hydrochloric acid solution or a 1:1 nitric acid solution. (Nitric acid is best when testing for lead or other metals.)
4. Rinse well with deionized water. For chromium, 12–15 rinses may be necessary. When determining ammonia and Kjeldahl nitrogen, the rinse water must be ammonia-free.
5. Air dry. Protect the glassware from fumes and other sources of contamination during storage.

Use chromic acid or chromium-free substitutes to remove organic deposits from glass containers. Afterward, rinse thoroughly with water to remove all traces of chromium.

Avoid introducing metal contaminants from containers, distilled water or membrane filters.

3.1.1.3 Sample splits

Samples must often be split or divided into separate containers for intra- or inter-laboratory use in studies, confirmation, alternative techniques or maintaining additional sample for reference and stability studies.

It is very important that sample splits be done correctly:

- Collect a large volume of sample in a single container and transfer to smaller containers; do not fill the smaller containers individually from the water source.
- Thoroughly mix samples containing particulates or solids before splitting so that all the splits are homogeneous.
- If the sample requires filtering before analysis or storage, filter the entire sample before splitting.
- Use the same kind of container for all the splits.
- Analyze biologically active splits on the same day or as close to the same day as is possible.
- Preserve all splits in the same way; if this is not done, the differing methods must be fully documented.
- When testing for volatile contaminants, fill containers samples to overflowing and cap carefully. Do not leave any headspace or air in the container.

3.1.2 Storage and preservation

Because chemical and biological processes continue after collection, analyze the sample as soon as possible. This also reduces the chance for error and minimizes labor. When immediate analysis is not possible, the sample must be preserved. Preservation methods include pH control, chemical addition, refrigeration and freezing.

Comparison of international drinking water and FDA bottled water guidelines presents an overview of preservation methods and holding times for specific procedures.

Preserve aluminum, cadmium, chromium, cobalt, copper, iron, lead, nickel, potassium, silver and zinc samples for at least 24 hours as follows:

1. Add one Nitric Acid Solution Pillow 1:1 (Catalog No. 254098) per liter of sample.
2. Check the pH with pH indicator paper or a pH meter to assure the pH is 2 or less. Add additional pillows if necessary.
3. Adjust the sample pH prior to analysis by raising the pH to 4.5 with Sodium Hydroxide Standard Solution, 1 N or 5 N.

3.1.3 Correcting for volume additions

When using a large volume of preservative or neutralizer, account for dilution by the acid added to preserve the sample and/or the base used to adjust the pH to the range of the procedure. Make this correction as follows:

1. Determine the volume of initial sample, the volume of acid and base added and the total final volume of the sample.
2. Divide the total volume by the initial volume.
3. Multiply the test result by this factor.

Example:
A one-liter sample was preserved with 2 mL of nitric acid. It was neutralized with 5 mL of 5 N sodium hydroxide. The result of the analysis procedure was 10.00 mg/L. What is the volume correction factor and correct result?

1. Total Volume = 1000 mL + 2 mL + 5 mL = 1007 mL

2. $\frac{1007}{1000}$ = 1.007 = volume correction factor

$10.0 \text{ mg/L} \times 1.007 = 10.07 \text{ mg/L}$ = correct result

Table 8 Required containers, preservation techniques and holding times [1]

Parameter name	Container[2]	Preservation[3,4]	Maximum holding time[5]
Bacterial tests			
Coliform, fecal and total	P, G	Cool, 4 °C, 0.008% $Na_2S_2O_3$	6 hours
Fecal streptococci	P, G	Cool, 4 °C, 0.008% $Na_2S_2O_3$	6 hours
Aquatic toxicity tests			
Toxicity, acute and chronic	P, G	Cool, 4 °C	36 hours
Chemical tests			
Acidity	P, G	Cool, 4 °C	14 days
Alkalinity	P, G	Cool, 4 °C	14 days
Ammonia	P, G	Cool, 4 °C, H_2SO_4 to pH<2	28 days
Biochemical oxygen demand (BOD)	P, G	Cool, 4 °C	48 hours
Boron	P, PFTE or quartz	HNO_3 to pH<2	6 months
Bromide	P, G	None required	28 days
Biochemical oxygen demand, carbonaceous	P, G	Cool, 4 °C	48 hours
Chemical oxygen demand	P, G	Cool, 4 °C, H_2SO_4 to pH<2	28 days
Chloride	P, G	None required	28 days
Chlorine, total residual	P, G	None required	Analyze immediately
Color	P, G	Cool, 4 °C	48 hours
Cyanide, total and amenable to chlorination	P, G	Cool, 4 °C, NaOH to pH>12, 0.6 g ascorbic acid[6]	14 days[7]
Fluoride	P	None required	28 days
Hardness	P, G	HNO_3 to pH<2, H_2SO_4 to pH<2	6 months
Hydrogen ion (pH)	P, G	None required	Analyze immediately
Kjeldahl and organic nitrogen	P, G	Cool 4 °C, H_2SO_4 to pH<2	28 days
Metals[8]			
Chromium VI	P, G	Cool, 4 °C	24 hours
Mercury	P, G	HNO_3 to pH<2	28 days
Metals, except boron, chromium VI and mercury	P, G	HNO_3 to pH<2	6 months
Nitrate	P, G	Cool, 4 °C	48 hours
Nitrate-nitrite	P, G	Cool 4 °C, H_2SO_4 to pH<2	28 days
Nitrite	P, G	Cool, 4 °C	48 hours

Table 8 Required containers, preservation techniques and holding times (continued)[1]

Oil and grease	G	Cool, 4 °C, HCl or H2SO4 to pH<2	28 days
Organic Carbon	P, G	Cool, 4 °C, HCl or H2SO4 or H3PO4 to pH<2	28 days
Orthophosphate	P, G	Filter immediately; Cool, 4 °C	48 hours
Oxygen, dissolved probe	G Bottle and top	None required	Analyze immediately
Winkler	G Bottle and top	Fix on site and store in dark	8 hours
48. Phenols	G only	Cool 4 °C, H2SO4 to pH<2	28 days
Phosphorus, elemental	G	Cool, 4 °C	48 hours
Phosphorus, total	P, G	Cool, 4 °C, H2SO4 to pH<2	28 days
Residue, Total	P, G	Cool, 4 °C	7 days
Residue, Filterable	P, G	Cool, 4 °C	7 days
Residue, Nonfilterable (TSS)	P, G	Cool, 4 °C	7 days
Residue, Settleable	P, G	Cool, 4 °C	48 hours
Residue, Volatile	P, G	Cool, 4 °C	7 days
Silica	P, PFTE or quartz	Cool, 4 °C	28 days
Specific Conductance	P, G	Cool, 4 °C	28 days
Sulfate	P, G	Cool, 4 °C	28 days
Sulfide	P, G	Cool 4 °C, add zinc acetate plus sodium hydroxide to pH>9	7 days
Sulfite	P, G	none required	Analyze immediately
Surfactants	P, G	Cool, 4 °C	48 hours
Temperature	P, G	None required	Analyze immediately
Turbidity	P, G	Cool, 4 °C	48 hours

[1] This table was adapted from Table II in the *Code of Federal Regulations*, July 1, 2000, Title 40, Part 136.3 (40 CFR 136.3), pages 23–25. Most organic tests are not included.

[2] Polyethylene (P) or glass (G) or PTFE Teflon

[3] Sample preservation should be performed immediately upon sample collection. For composite chemical samples each portion should be preserved at the time of collection. When use of an automated sampler makes it impossible to preserve each portion, then chemical samples may be preserved by maintaining at 4 °C until compositing and sample splitting is completed.

[4] When any sample is to be shipped by common carrier or sent through United States Mails, it must comply with the *Department of Transportation Hazardous Material Regulations* (49 CFR Part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO_3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

[5] Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still be considered valid. Samples may be held for longer periods only if the permittee or monitoring laboratory has data on file to show that the specific types of samples under study are stable for the longer time and has received a variance from the Regional Administrator under §136.3(e). Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See §136.3(e) for details. The term “analyze immediately” usually means within 15 minutes or less after sample collection.

[6] Should only be used in the presence of residual chlorine.

[7] Maximum holding time is 24 hours when sulfide is present. Optionally all samples may be tested with lead acetate paper before pH adjustments in order to determine if sulfide is present. If sulfide is present, it can be removed by the addition of cadmium nitrate powder until a negative spot test is obtained. The sample is filtered and then NaOH is added to pH 12.

[8] Samples should be filtered immediately on-site before adding preservative for dissolved metals.

3.1.4 Checking for accuracy and precision

Accuracy defines the closeness of a test result to the true value. Precision defines the closeness of repeated measurements to each other. Although precise results suggest accuracy, they can be inaccurate. Both the accuracy and the precision of test results can be evaluated by using standard additions or standard solutions.

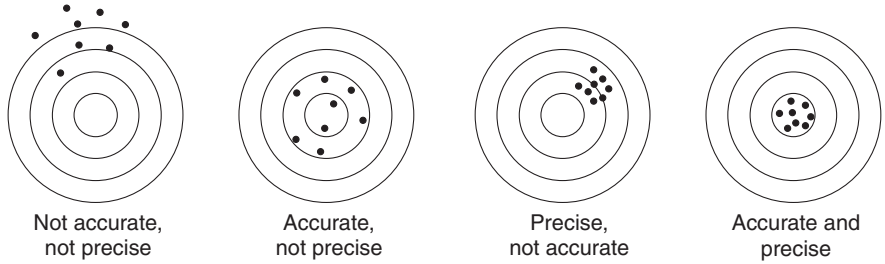

Figure 10 Precision and accuracy illustrated

3.1.5 Standard solutions

A standard solution may be ordered as a prepared reagent or it may be made in the laboratory. It is a solution of a known composition and concentration. The accuracy of the analysis system may be checked by using a standard solution in place of the sample water in a procedure.

3.1.6 Standard additions

Standard Additions is a common technique for checking test results. Other names are “spiking” and “known additions.” The technique can test for interferences, bad reagents, faulty instruments and incorrect procedures.

Perform Standard Additions by adding a measured small amount of a standard solution to the sample and repeating the test. Use the same reagents, equipment and technique. The result should be about 100% recovery. If not, there is an identifiable problem.

If Standard Additions works for the test, a Standard Additions Method section will be in the procedure under Accuracy Check. Follow the detailed instructions given.

If the result is about 100% recovery for each addition, everything is working properly and the results are correct.

If the result is not approximately 100% recovery for each addition, a problem exists. To determine if the cause is an interference, repeat the Standard Additions using deionized water as the sample. If the result is approximately 100% recovery for each addition, an interference exists.

If results did show good recoveries with the deionized water, use the following checklist to find the problem:

1. Check that the procedure is followed exactly:

- **a.** Are the correct reagents used in the correct order?
- **b.** Is the correct time allowed for color to develop?
- **c.** Is the correct glassware being used?
- **d.** Is the glassware clean?

e. Does the test need a specific sample temperature?

f. Is the sample pH in the correct range?

Refer to the written procedure to answer these questions.

2. Check the performance of the instrument per instructions in the user manual.

3. Check the reagents. Repeat the Standard Additions using new, fresh reagents. If results are good, the original reagents were faulty.

4. If nothing else is wrong, the standard is almost certainly defective. Repeat the Standard Additions with a new standard.

If the problem is still uncertain, call Technical Customer Support at 800-227-4224 (U.S.A.) or 303-669-3050 for assistance.

3.1.7 Troubleshoot a test when results are in doubt

If the results from any chemistry are in doubt, troubleshoot as follows:

1. Run a proof-of-accuracy check. Take a standard solution, which has a known concentration, through the same steps as the original sample. Include sampling and storage, digestion and colorimetric determination, if applicable. If the results of the standard solution check are correct, skip to step **4** below. If there is a variation in the expected results, go to step **2**.

2. If the standard solutions check does not match the expected results, check the instrument set-up and method procedure as follows:

a. Verify that the correct program number for the test being performed is selected.

b. Verify that the units of concentration of the standard match the displayed units. (One of the alternative forms of the analyte may be in the display.) For example: Molybdenum may be shown as Mo instead of MoO_4.

c. Verify that the sample cells specified in the procedures are used.

d. Verify that the reagents are correct for the sample size being analyzed.

e. Verify that the reagent blank value stored is for the current procedure. It may be from a previous lot of reagents and therefore not representative of the current reagent lot.

f. Verify the calibration curve adjustment (Standard Adjust) currently in use. The factory-stored default calibration should be used initially to check the standard.

g. Verify that the dilution factor option is correct.

If the instrument setup is correct and the method procedure specifics are being followed correctly, go to step **3**.

3. If the standard solution check does not match the expected results, check the reagents used in the test and the analytical technique as follows:

a. Determine the age of the reagents used in the test. Many factors affect reagent shelf life (i.e., storage temperature, storage conditions, microbial contamination). Replace suspect reagents and run the standards check again.

b. Run a deionized or distilled water blank through the entire process; include sampling and storage, digestion and colorimetric determination. Some chemicals will add a small amount of color to a test; this is not considered unusual. However, color development in excess of 10% of the range of the test may indicate a problem with one of the reagents or the dilution water.

c. To troubleshoot the procedure, delete the parts one by one. First, using the standard solution, omit preservation and storage, doing only digestion and colorimetry. If this analysis is correct, examine the procedure used to store the

sample. Make sure that it is the procedure prescribed for the chosen parameter. If the sample is acidified for storage, be sure the correct acid is used and the sample is adjusted to the proper pH level before testing.

If the standards check is still incorrect, run the standard on just the colorimetry. If the results are correct, examine the digestion procedure. Make sure that the amount of reagents used and the pH after the digestion are correct for the procedure. (See the procedure for the parameter in question.)

4. If the standard solution gives a correct value, but the results of the sample measurement are questionable, there may be an interference in the sample. To check for an interference:

a. Spike the sample. Use a standard addition test instead of a standard solution test to include any possible interferences.

b. To two cells containing fresh sample water, add an amount of standard equal to two times the concentration of the sample.

c. Process both samples using the same reagents, instruments and technique. The spiked sample should show an increase equal to the amount of standard added.

d. Calculate percent recovery as shown below. Ideally, the results should be 100%, with results from 90 to 110% considered acceptable. Refer to the procedure notes for possible interferences and ways to eliminate them.

e. Run a series of dilutions on the sample. Make sure the sample is within the range of the test. A sample out of range for the method may give erroneous results because of under- or over-development of the color, excess turbidity or even sample bleaching. Run a series of dilutions to check for this possibility.

f. Because it may not be feasible to determine the cause of the interference, diluting the sample past the point of interference is often the most economical and efficient means of getting the correct result. If it is not possible to dilute out an interference without diluting out the parameter to be measured, use a different method, such as a different chemistry or an ion-selective electrode to measure the parameter.

Calculating percent recovery:

1. Measure the unknown sample concentration.

2. Calculate the theoretical concentration of the spiked sample using the following formula:

$$\text{Theoretical Concentration} = \frac{(C_u \times V_u) + (C_s \times V_s)}{V_u + V_s}$$

Where:

C_u = measured concentration of the unknown sample
V_u = volume of the unknown sample
C_s = concentration of the standard
V_s = volume of the standard

3. Measure the spiked sample concentration.

4. Divide the spiked sample concentration by the theoretical concentration and multiply by 100.

For example:
A sample was tested for manganese and the result was 4.5 mg/L. A separate 97-mL portion of the same sample was spiked with 3 mL of a 100 mg/L standard solution of manganese. This spiked solution was tested again for manganese using the same method. The result was 7.1 mg/L.

The theoretical concentration of the spiked sample is:

$$\frac{(4.5\ \text{mg/L} \times 97\ \text{mL}) + (100\ \text{mg/L} \times 3\ \text{mL})}{97\ \text{mL} + 3\ \text{mL}} = 7.4\ \text{mg/L}$$

The percent spike recovery is:

$$\frac{7.1\ \text{mg/L}}{7.4\ \text{mg/L}} \times 100 = 96\%$$

USEPA calculation
The USEPA requires a more stringent calculation for percent recovery. This formula calculates the percent recovery only for the standard added to the spiked sample and yields a lower value than the above calculation. A complete explanation for the USEPA formula is in *USEPA Publication SW-846*. The USEPA percent recovery formula is:

$$\%R = \frac{100(X_s - X_u)}{K}$$

Where:

X_s = measured value of the spiked sample
X_u = measured value for the unspiked sample, adjusted for the dilution of the spike volume
K = known value of the spike in the sample

Example:
A sample measures 10 mg/L. A separate 100-mL portion of the sample was spiked with 5 mL of a 100-mg/L standard solution. The spiked solution was measured by the same method as the original sample. The result was 13.7 mg/L.

$$X_s = 13.7\ \text{mg/L}$$

$$X_u = \frac{10\ \text{mg/L} \times 100\ \text{mL}}{105\ \text{mL}} = 9.5\ \text{mg/L}$$

$$K = \frac{5\ \text{mL} \times 100\ \text{mg/L}}{105\ \text{mL}} = 4.8\ \text{mg/L}$$

$$\%R = \frac{100 \times (13.7\ \text{mg/L} - 9.5\ \text{mg/L})}{4.8\ \text{mg/L}} = 88\%$$

Acceptable percent recovery values are 80–120%.

3.1.8 Adjusting the standard curve

Spectrophotometers typically have many programs permanently installed in memory. Many programs include a pre-programmed calibration curve. Each curve is the result of an extensive calibration performed under ideal conditions and is normally adequate for most testing. Deviations from the curve can occur from using compromised testing reagents, defective sample cells, incorrect test procedure, incorrect technique or other correctable causes. Interfering substances or other causes may be beyond the analyst's control.

In some situations, using the pre-programmed curve may not be convenient:

- Running tests where the reagents are highly variable from lot to lot.
- Running tests where frequent calibration curve checks are required.
- Testing samples which give a consistent test interference.
- Consider the following before adjusting the calibration curve:

- Will future test results be improved by adjusting the curve?
- Are interfering substances consistent in all the samples tested?
- Estimated detection limit, sensitivity, precision and test range information provided with the procedure may not apply to an adjusted curve calibration.

The calibration curves can be adjusted by following the steps found in the test procedure. Generally, add test reagents to a blank and standard solution. Working carefully is important. After the adjustment, it is wise to run standard solutions of several concentrations to make sure the adjusted curve is satisfactory. Performing standard additions on typical samples may also help determine if the adjusted curve is acceptable.

Adjusting a measurement is a two-step process. First, the instrument measures the sample using the pre-programmed calibration. Second, it multiplies this measurement by an adjustment factor. The factor is the same for all concentrations. The instrument will remember the factor until the program is exited and will display the standard adjustment icon when it is used. Return to the pre-programmed curve any time by selecting the original stored program from the main menu.

3.2 Interferences

Interferences are contaminants in a sample that are capable of causing changes in color development, turbidity or unusual colors and odors, thereby creating errors in the results. A list of common interferences is included in each procedure. Reagents are formulated to eliminate many interferences; remove others by pretreating the sample as instructed in the procedure.

Test strips are available for many of the common interferences. These can be conveniently used to screen samples for the presence of interferences.

When test results appear inaccurate, develop an unexpected color or develop an unusual odor or turbidity, repeat the test on a sample diluted with deionized water. (See *Sample dilution*.) Correct the results for the dilution and compare them with those from the original test. If they differ significantly, make a second dilution and check it against the first. Repeat the dilutions until the same result (after volume corrections) is achieved twice in succession.

For more information on interferences, see *Standard additions*. The *APHA Standard Methods* book, an excellent reference for water analysis, also covers interferences in the “General Introduction.”

pH interference

Chemical reactions are often pH dependent. Reagents contain buffers to adjust the pH of the sample to the correct range. However, the reagent buffer may not be strong enough for samples that are highly buffered or have an extreme pH.

The *Sampling and Storage* section of each procedure gives the pH range for that test.

Before testing, adjust the sample to the proper pH as instructed in the procedure or by following these steps:

1. Measure the pH of the analyzed sample with a pH meter.

   Note: *Use pH paper when testing for chloride, potassium or silver to avoid contamination.*

2. Prepare a reagent blank using deionized water as the sample. Add all reagents called for in the procedure. Timer sequences, etc., may be ignored. Mix well.
3. Measure the pH of the reagent blank with a pH meter.
4. Compare the pH values of the analyzed sample with the reagent blank.

5. If there is little difference in the values of the analyzed sample and the reagent blank, then pH interference is not the problem. Follow the *Accuracy Check* for the specific procedure to more clearly identify the problem.
6. If there is a large difference between the value of the analyzed sample and the reagent blank, adjust the sample pH to the value of the reagent blank. Adjust the sample pH to this same pH for all future samples before analysis. Use the appropriate acid, usually nitric acid, to lower the pH. Use the appropriate base, usually sodium hydroxide, to raise the pH. Adjust the final result for any dilution caused by adding acid or base; see *Correcting for volume additions*.
7. Analyze the sample as before.
8. Some purchased standards may be very acidic and will not work directly with test procedures. Adjust the pH of these standards as described above. Adjust the final concentration of the standard for the dilution. The standard solutions suggested in the procedures are formulated so that no pH adjustment is necessary.

3.3 Method performance

3.3.1 Estimated detection limit (EDL)

Ranges for chemical measurements have limits. The lower limit is important because it determines whether a measurement is different from zero. Many experts disagree about the definition of this detection limit and determining it can be difficult. The *Code of Federal Regulations* (40 CFR, Part 136, Appendix B) provides a procedure to determine the "Method Detection Limit" or MDL. The MDL is the lowest concentration that is different from zero with a 99% level of confidence. A measurement below this MDL is highly suspect.

The MDL is not fixed; it varies for each reagent lot, instrument, analyst, sample type, etc. Therefore, a published MDL may be a useful guide, but is only accurate for a specific set of circumstances. Each analyst should determine a more accurate MDL for each specific sample matrix using the same equipment, reagents and standards that will routinely be used for measurements.

A sensitivity value (concentration change equivalent to an absorbance change of 0.010 abs) is provided as an estimate of the lower detection limit of each test. The sensitivity value may be treated as an EDL for the purposes of MDL determination. It can be considered a good starting concentration when determining a MDL. **Do not use the EDL as the MDL**. The conditions for MDL determination must be exactly the same as the conditions used for analysis. The EDL may be useful to the analyst as a starting point in determining a MDL or as a way to compare methods. Measurements below the EDL may also be valuable because they can show a trend, indicate the presence of analyte and/or provide statistical data. However, these values have a large uncertainty.

3.3.2 Method detection limit (MDL)

This method is in accordance with the USEPA definition in 40 CFR, Part 136, Appendix B in the 7-1-94 edition. The USEPA defines the method detection limit (MDL) as the minimum concentration that can be determined with a 99% level of confidence that the true concentration is greater than zero. Since the MDL will vary from analyst to analyst, it is important that the MDL be determined under actual operating conditions.

The procedure for determining MDL is based on replicate analyses at a concentration 1 to 5 times the estimated detection limit. The MDL value is calculated from the standard deviation of the replicate study results multiplied by the appropriate ***t*** value for a 99% confidence interval. For this definition, the MDL does not account for variation in sample composition and can only be achieved under ideal conditions.

1. Estimate the detection limit. Use the sensitivity value stated in the *Method Performance* section of the analysis procedure.
2. Prepare a laboratory standard of the analyte, 1 to 5 times the estimated detection limit, in deionized water that is free of the analyte.
3. Analyze at least seven portions of the laboratory standard and record each result.
4. Calculate the average and the standard deviation (*s*) of the results.
5. Compute the MDL using the appropriate ***t*** value (see table below) and the standard deviation value:

   MDL = ***t*** x *s*

Number of test portions	*t* Value
7	3.143
8	2.998
9	2.896
10	2.821

For example:
The EDL for measuring iron using an iron test is 0.003 mg/L. An analyst accurately prepared 1 liter of a 0.010 mg/L (about 3x the EDL) laboratory standard by diluting a 10-mg/L iron standard in iron-free deionized water.

Eight portions of the standard were tested according to the FerroZine method with the following results:

Sample #	Result (mg/L)
1	0.009
2	0.010
3	0.009
4	0.010
5	0.008
6	0.011
7	0.010
8	0.009

Using a calculator program, the average concentration = 0.010 mg/L and the standard deviation (*s*) = 0.0009 mg/L.

Based on the USEPA definition, calculate the MDL as follows:

MDL for iron test = 2.998 (***t***) x 0.0009 (*s*)
MDL = 0.003 mg/L (agrees with initial estimate)

Note: *Occasionally, the calculated MDL may be very different from the estimate of the detection limit. To test how reasonable the calculated MDL is, repeat the procedure using a standard near the calculated MDL. The average result calculated for the second MDL derivation should agree with the*

initial calculated MDL. Refer to 40 CFR, Part 136, Appendix B (7-1-94), pages 635–637 for detailed procedures to verify the MDL determination.

Run a laboratory blank containing deionized water without analyte through the test procedure to confirm that the blank measurement is less than the calculated MDL. If the blank measurement is near the calculated MDL, repeat the MDL procedure using a separate blank for analysis for each portion of standard solution analyzed. Subtract the average blank measurement from each standard and use the corrected standard values to calculate the average and standard deviation used in the MDL.

3.3.3 Precision

Every chemical measurement has some degree of uncertainty. The quality of the entire calibration curve determines the precision.

Uncertainty in chemical measurements may be due to systematic errors and/or random errors. A systematic error is a mistake that is always the same for every measurement made. For example, a blank can add to each measurement for a specific compound, giving consistently high results (a positive bias). Random errors are different for every test and can add either a positive or negative variation in response. Random errors are most often caused by variation in analytical technique. Even with reliable reagents developed to eliminate systematic errors, response variation occurs in all chemical measurements.

3.3.4 Estimating precision

The method performance section in each procedure provides an estimate of test precision. Most procedures use a *replicate analysis* estimate, based on real data. For precision determined in this manner, the 95% confidence interval of the distribution is reported.

In replicate analysis, the chemist prepares a specific concentration of the analyte in a deionized water matrix. The standard is analyzed seven individual times on a single instrument. The standard deviation is calculated and the 95% confidence interval of the distribution is reported in the method. The reported value provides an estimate of the “scatter” of results at a particular point in the calibration curve.

Precision estimates are based on a deionized water matrix. Precision on real samples with varying matrices can be quite different from these estimates.

If the concentration obtained from running a standard solution is not as expected, refer to *Troubleshoot a test when results are in doubt*.

3.3.5 Sensitivity

The definition of the sensitivity of a method is a change in concentration (ΔConcentration) for a 0.010 change in absorbance (ΔAbs).

Use sensitivity when comparing different methods. For example, between three methods for determining iron:

Iron Analysis Method	Portion of curve	ΔAbs	ΔConcentration
FerroVer	Entire range	0.010	0.022 mg/L
FerroZine	Entire range	0.010	0.009 mg/L
TPTZ	Entire range	0.010	0.012 mg/L

Notice that the FerroZine method has the greatest sensitivity of the three methods because it will measure the smallest change in concentration. The technical definition of sensitivity comes from a calibration curve with Abs on the x-axis and concentration on the y-axis.

1. If the calibration is a line, the sensitivity is the slope of the line multiplied by 0.010.
2. If the calibration is a curve, the sensitivity is the slope of the tangent line to the curve at the concentration of interest multiplied by 0.010.

The sensitivity value is also used as an estimate of the lower limit of the test. The value may be used as a starting point in determining MDL.

3.4 Prepare a calibration curve

Note: *Calibration curves are recommended when performing tests on different instruments or where required by a regulator.*

1. Prepare five or more standards of known concentration that cover the expected range of the test. Run tests as described in the procedure on each prepared standard. Then pour the customary volume of each known solution into a separate clean sample cell of the type specified for the instrument.
2. Select the proper wavelength. Standardize (zero) the instrument using an untreated water sample or a reagent blank, per procedure instructions.
3. Measure and record the absorbance of the known solutions within the time constraints detailed in the procedure. To use absorbance vs. concentration, see the section on *Absorbance versus concentration calibration*.

3.4.1 Absorbance versus concentration calibration

If absorbance values are measured, plot the results on linear graph paper. Plot the absorbance value on the vertical axis and the concentration on the horizontal axis.

Plot increasing absorbance values from bottom to top. Plot increasing concentration values from left to right. Values of 0.000 absorbance units and 0 concentration will begin at the bottom left corner of the graph. A calibration table can be extrapolated from the curve or the concentration values can be read directly from the graph. Or, determine an equation for the line using the slope and y-intercept.

Alternately, use the User Program software in the spectrophotometer or a curve-fitting program such as Excel to calculate the calibration curve.

3.5 Adapt procedures to other spectrophotometers

Test procedures may be used with more than one spectrophotometer, if calibration curves are made that convert absorbance to concentration. Regardless of the spectrophotometer used, prepare the sample and calibration standards following the procedure and use the optimum wavelength used in the procedure.

To calibrate for a given analyte, a series of standards are prepared and measured to establish the calibration curve. The absorbance vs. concentration is plotted as described in the section on *Absorbance versus concentration calibration*. Points on the graph are connected with a smooth line (curved or straight). If necessary, use the curve to make a calibration table.

3.5.1 Select the best wavelength

When developing a new procedure or using procedures that are sensitive to wavelength, select the wavelength where the instrument gives the greatest absorbance (see the *Select the best wavelength* figure). Because procedures are usually developed to use the best wavelength for the test, selecting the wavelength is not necessary for most stored procedures.

3.5.1.1 Select the best wavelength on a spectrophotometer:

Note: *When available, use of the Scan function is the easiest way to find the optimum wavelength.*

1. Refer to the user manual for specific instructions for wavelength adjustments.
2. Select single wavelength adjustment.
3. Enter a wavelength in the range of interest.

Note: *Sample color provides a good indication of what wavelength region to use. A yellow solution absorbs light in the 400–500 nm region. A red solution absorbs light between 500–600 nm. A blue solution absorbs light in the 600–700 nm range.*

4. Prepare the sample and blank for analysis. Fill the appropriate sample cells with the blank and the reacted sample solution.
5. Place the blank in the cell holder. Zero the instrument.
6. Place the prepared sample into the cell holder. Read the absorbance level.
7. Increase the wavelength so it is at least 100 nm greater than the range of interest. Re-zero as in step **5**. Measure and record the absorbance of the sample.
8. Repeat, decreasing the wavelength by 50 nm. Re-zero, then measure and record the absorbance at each increment. Continue this process throughout the wavelength range of interest. Note the wavelength of greatest absorbance (see the *Example* in Table 9).

Table 9 Example

Wavelength	Absorbance
550 nm	0.477
500 nm	0.762
450 nm	0.355
400 nm	0.134

9. Adjust the wavelength to 50 nm more than the highest absorbance point on the initial search (step **8**). Re-zero as in step **5**.

Measure and record the absorbance. Repeat, decreasing the absorbance in 5-nm steps. Re-zero, then measure and record the absorbance at each increment. Continue until the entire range of interest is measured (see the *Example* in Table 10).

Table 10 Example

Wavelength	Absorbance
520 nm	0.748
515 nm	0.759
510 nm	0.780
505 nm	0.771
500 nm	0.771
495 nm	0.651
490 nm	0.590

Check to be sure there is enough difference in absorbance between samples with low and high analyte concentrations by measuring two sample solutions that contain the expected low and high concentrations of analyte at the optimum wavelength. The change in absorbance caused by increases/decreases in concentration depends on the sensitivity of the procedure and the chemistry. Chemistries with small absorbance changes are less sensitive, but tend to have larger ranges. Chemistries with large absorbance changes are more sensitive, but tend to have smaller ranges.

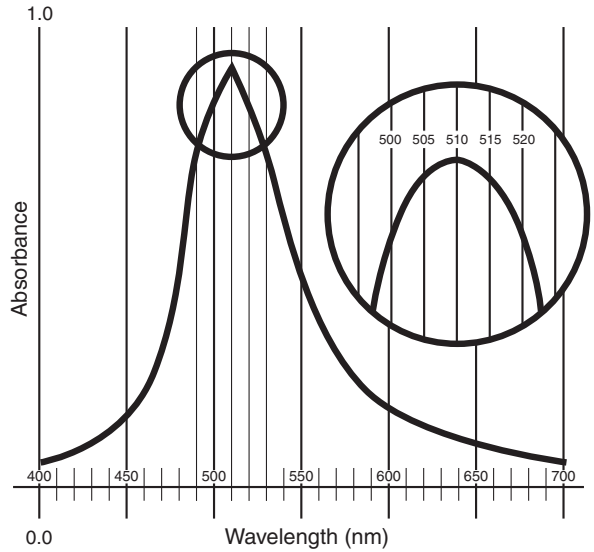

Figure 11 Select the best wavelength

Section 4 Sample Pretreatment by Digestion

Several procedures require sample digestion before determining total metal content. Digestion uses acid and heat to break organo-metallic bonds and free ions for analysis.

4.1 USEPA-approved digestions

For USEPA reporting, USEPA-approved digestions are required. There are two methods for metals analysis: mild and vigorous.

4.1.1 USEPA mild digestion

1. Acidify the entire sample at the time of collection with concentrated nitric acid by adding 5 mL of acid per liter (or quart) of sample.
2. Transfer 100 mL of well-mixed sample to a beaker or flask. Add 5 mL of distilled 1:1 hydrochloric acid (HCl).
3. Heat using a steam bath or hot plate until the volume has been reduced to 15–20 mL. Do not boil.
4. Filter the sample to remove any insoluble material.
5. Adjust the pH of the digested sample to a pH of 4 by adding 5.0 N Sodium Hydroxide Standard Solution a drop at a time. Mix thoroughly and check the pH after each addition.
6. Quantitatively transfer the sample to a 100-mL volumetric flask and dilute to volume with deionized water. Continue with the procedure.
7. Process a reagent blank to match the sample.

4.1.2 USEPA vigorous digestion

For some samples mild digestion will not be sufficient. Use a vigorous digestion to make sure that all the organo-metallic bonds are broken.

1. Use redistilled 1:1 Nitric Acid Solution to acidify the entire sample to a pH of less than two. Do not filter the sample before digestion.
2. Transfer an appropriate sample volume (see the table on *Vigorous digestion volumes*) into a beaker and add 3 mL of concentrated redistilled nitric acid.
3. Place the beaker on a hot plate and evaporate to near dryness, making certain the sample does not boil.
4. Cool the beaker and add another 3 mL of the concentrated re-distilled nitric acid.
5. Cover the beaker with a watch glass and return it to the hot plate. Increase the temperature of the hot plate so that a gentle reflux occurs. Add additional acid, if necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change color or appearance with continued refluxing).

6. Again, evaporate to near dryness (do not bake) and cool the beaker. If any residue or precipitate results from the evaporation, add redistilled 1:1 hydrochloric acid (5 mL per 100 mL of final volume). See the table on *Vigorous digestion volumes*.
7. Warm the beaker. Adjust the sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution; mix thoroughly and check the pH after each addition.
8. Quantitatively transfer the sample to a volumetric flask and dilute to volume with deionized water. See the *Vigorous digestion volumes* table for the suggested final volume.
9. Multiply the result by the correction factor in the *Vigorous digestion volumes* table.
10. Process a reagent blank to match the sample.

Table 11 Vigorous digestion volumes

Expected metal concentration	Suggested sample vol. for digestion	Suggested volume of 1:1 HCl	Suggested final volume after digestion	Correction factor
1 mg/L	50 mL	10 mL	200 mL	4
10 mg/L	5 mL	10 mL	200 mL	40
100 mg/L	1 mL	25 mL	500 mL	500

4.2 General Digesdahl digestion

Many samples may be digested using the Digesdahl Digestion Apparatus (Catalog. No. 2313020). It is designed to digest samples such as oils, wastewater, sludges, feeds, grains, plating baths, food and soils. In this procedure the sample is oxidized by a mixture of sulfuric acid and hydrogen peroxide. Digestion of a dry sample requires less than ten minutes, while liquid samples require about 1 minute/mL. The digestion is done in a special flat-bottomed, 100-mL volumetric flask. Aliquots (sample portions) are taken for analysis using colorimetric methods.

Procedures for digestion using the Digesdahl Digestion Apparatus are based on the type and form of the sample and are found in the *Digesdahl Digestion Apparatus Instruction Manual*, which is included with each Digesdahl Digestion Apparatus.

Digesdahl digestion is a process that yields a digest suitable for the determination of metals, total phosphorus and total Kjeldahl nitrogen (TKN). It is faster than traditional methods, but yields comparable accuracy and precision. The digest can be used with colorimetric, turbidimetric or titrimetric tests.

Procedures for using the Digesdahl Digestion Apparatus vary with sample type. Sample types include food products, feeds, grains, wastewater sludges, plating baths, plant tissues, fertilizers, beverages and oils. Most procedures use a two-phase digestion process involving concentrated sulfuric acid and 50% hydrogen peroxide. Sulfuric acid dehydrates and chars the sample. Hydrogen peroxide is added through the capillary flow funnel to complete decomposition. The analyst controls the digestion time (exposure to the hydrogen peroxide) by varying the volume of hydrogen peroxide used.

Some samples are more difficult to digest completely. For example, resistant or refractory materials such as nicotinic acid require several minutes of continued peroxide digestion after clearing to obtain 100% nitrogen recovery. To make sure complete sample digestion, consider variables such as sample size, solution temperature and sample contamination. Refer to the *Digesdahl Manual* (Catalog No. 2313018) for complete information.

4.2.1 Frequently asked questions regarding digestion procedures

This section provides answers to common questions about digestion.

The reading on my instrument is over-range. What should I do?
The concentration range tables found in digestion procedures are only guidelines. Take a smaller analysis volume and repeat the procedure. Record the new analysis volume and use it in the calculation.

Do I have to prepare a reagent blank each time I use reagents with the same lot number?
To decide, first determine the reading of the reagent blank by zeroing the instrument with deionized or distilled water. If the reagent blank has an insignificant concentration reading and the reagents have the same lot number, a reagent blank does not have to be prepared every time. If the reagent blank shows a reading, either analyze it daily or subtract the reading from the sample reading. If a reagent blank is not run daily, zero the instrument with deionized water.

Do I have to follow the exact sample amount and the exact analysis volume given for each procedure?
The sample amount and the analysis volume for each procedure are only suggested guidelines. Digest any aqueous solution or suspension sample amount up to 40 mL. Solid or organic liquid samples require less than 0.5 g of anhydrous material—as a routine practice, 0.25 g of sample is used.

How can I refine the initial amount of sample required for digestion and the analysis volume to be used?
The amount of sample to be digested is a critical aspect of the digestion. The aliquot size of the digest to be used in the analysis is also very important. Tables are provided in each method for determining amount of initial sample to be digested. In order to optimize the specific test being performed, the following equations have been developed. Before using these equations, please refer to the manual specifications for the sample type.

To use the equations, first determine the approximate concentration (in ppm or mg/L or mg/kg). Next determine the range of the colorimetric test being used (e.g., 0–50 mg/L) and the midpoint of the test range. (This midpoint range is optimum but can be lowered to accommodate very low sample concentrations). The midpoint of the test range is determined by subtracting the lower limit of the range from the higher limit and dividing by 2.

After finishing these determinations, the following equation may be used:

$$A = \frac{B \times C \times D}{E \times F}$$

Where:

A = approximate concentration of sample
B = midpoint of colorimetric test range
C = final volume of digest
D = final volume of analysis
E = sample amount to digest
F = analysis volume of digest

Using algebra, the following two equations are obtained:

equation (1) $$E = \frac{B \times C \times D}{A \times F}$$

equation (2) $$F = \frac{B \times C \times D}{A \times E}$$

Both equations contain two unknown values, E and F and some trial and error may be required to achieve the optimum values.

Using equation (1): If analyzing for copper using the CuVer™ method with an initial sample containing approximately 150 ppm Cu, the amount of sample required for digestion and the aliquot volume to be used can be determined as follows:

Determine the test range. In this example, the test range is assumed to be 0–5.0 ppm and the midpoint is 2.5. When using the Digesdahl system, the final volume of digest is 100 mL and the procedure calls for a final analysis volume of 25 mL.

Therefore:

A = 150	B = 2.5	C = 100	D = 25	E and F are unknown

Substituting values into equation (1) gives:

$$E = \frac{2.5 \times 100 \times 25}{150 \times F} \quad \text{or} \quad E = \frac{41.7}{F}$$

Since CuVer Copper Reagent is pH sensitive, a small analysis volume (0.5 mL) is desired so that pH adjustment would not be necessary.

With this in mind, a 0.5-mL analysis volume would give:

$$E = \frac{41.7}{0.5} = 83.4 \text{ mL digestion sample amount}$$

Because the maximum digestion sample amount is 40 mL for Digesdahl digestions, a 0.5-mL analysis volume would not be acceptable for the range. (This is where trial and error is necessary). Next, try a 5.0-mL analysis volume and the equation gives:

$$E = \frac{41.7}{5.0} = 8.0 \text{ mL digestion sample amount}$$

(Round to the nearest whole number for ease of measure).

From the calculation, an 8.0 mL sample amount would be digested and a 5.0-mL analysis volume would be taken. A pH adjustment would be necessary before analysis.

Using equation (2): Equation (2) may be used when a minimum sample size is desired or when a sample has already been digested for one parameter (such as copper) and another parameter (such as zinc) also needs to be measured. Continuing the example for copper, above, a zinc test may also be performed. The undigested sample contains approximately 3 ppm zinc and the Zincon method is being used. The analysis volume can be determined as follows.

In this example, the Zincon method's test range is assumed to be 0–2.5 ppm so the midpoint of the range is 1.25. Therefore values are:

A = 3
B = 1.25
C = 100
D = 50
E = 8 (as determined above)

substituting:

$$F = \frac{1.25 \times 100 \times 50}{3 \times 8} = 260 \text{ mL}$$

This is an extreme example but it illustrates the need to compare the values of D and F to assure that the analysis volume (F) does not exceed the final analysis volume (D). If F exceeds D, the analysis cannot be done. A test with a more suitable range is necessary or a larger sample may be digested for this test. Care must also be taken to assure that the volume of digest taken for analysis (F) is greater than 0.1 mL because accurately pipetting less than 0.1 mL is difficult.

As a comparison, assume that the concentration of zinc is 75 ppm (A = 75 instead of 3) and substituting again will give:

$$F = \frac{1.25 \times 100 \times 50}{75 \times 8} = 10.5 \text{ mL}$$

In this case, the aliquot volume is less than the final analysis volume and analysis may be done as stated in the procedure.

Why is the factor in the calculation step either 75, 2500 or 5000 depending on the method used and where does the factor come from?
In all cases, the factor is a correction for sample dilution. For example, in some tests the factor is 2500. The Digesdahl digestion total volume is 100 mL, the analysis total volume is 25 mL and 100 x 25 = 2500. The mL units are not included with the factor because they cancel out in the formula.

How do I report my total concentration on a dry basis when I am analyzing a slurry?
The sample must be analyzed for moisture content. See the figure for *Determining dry basis weight*.

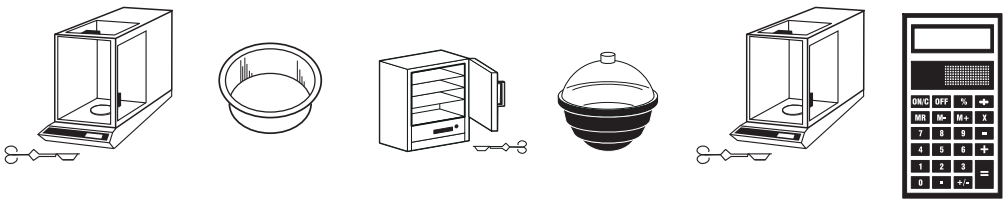

Figure 12 Determining dry basis weight

1 Weigh an aluminum dish and record the weight as “A”.	**3** Place the dish in the oven (103–105 °C) for two hours.	**5** Weigh the aluminum dish with oven-dried sample. Record as “C.” ***Note:*** *The oven-dried material generally is unsuitable for additional testing and should be discarded.*
2 Weigh out approximately 2 g of solid sample into the dish. Record the exact weight added as “B.”	**4** Cool to room temperature by placing in a desiccator	**6** Use the following formula to calculate the sample on a “dry basis.” Test result (dry basis) = $\frac{C - A}{B - A}$ ***Note:*** *Multiply the test result on an “as is” basis, by the factor above, to report as “dry basis.*

Table 12 Required appartaus for dry basis weight

Description	Unit	Catalog. No.
Balance, general laboratory, 50-g	each	2610300

Table 12 Required appartaus for dry basis weight (continued)

Desiccant, Drierite (without indicator)	454 g	2285901
Desiccator, vacuum (uses ceramic plate)	each	2088800
Dish, moisture determination, aluminum, 63 x 17.5 mm	100/pkg	2164000
Oven, laboratory		
120 VAC	each	1428900
or		
240 VAC	each	1428902
Tongs, crucible	each	56900

Table 13 Optional apparatus

Description	Unit	Catalog No.
Desiccator, without stopcock	each	1428500

4.2.2 Adjusting the pH

4.2.2.1 For a metals procedure

Note: *If analyzing aliquots smaller than 0.5 mL, pH adjustment is unnecessary.*

1. Determine the required volume of sample for analysis from the Sample and Analysis Volume Tables following each digestion procedure. Determine the required volume of sample for analysis from the Sample and Analysis Volume Tables following the digestion procedure. Pipet this volume into a mixing graduated cylinder.

   Note: *Some methods require pipetting into a volumetric flask or a regular graduated cylinder.*

2. Dilute to about 20 mL with deionized water.
3. Add one drop of 2,4 Dinitrophenol Indicator Solution (Cat. No. 186932).
4. Add one drop of 8 N Potassium Hydroxide (KOH) Standard Solution (Cat. No. 28232H), swirling between each addition, until the first flash of yellow appears (pH 3). If analyzing for potassium, use 5 N sodium hydroxide (Cat. No. 245026) instead. Do not use a pH meter if analyzing for potassium or silver.
5. Add one drop of 1 N KOH (Cat. No. 2314426). Stopper the cylinder and invert several times to mix. If analyzing for potassium, use 1 N sodium hydroxide instead.

   Note: *Use pH paper to make sure that the pH is 3. If it is higher than 4, do not readjust with acid; start over with a fresh aliquot.*

6. Continue to add 1 N KOH in this manner until the first permanent yellow color appears (pH 3.5–4.0).
7. View the cylinder from the top against a white background. Compare the cylinder to a second cylinder filled to the same volume with deionized water.

   Note: *High iron content will cause precipitation (brown cloud) which will co-precipitate other metals. Repeat this procedure with a smaller aliquot volume.*

8. Add deionized water to the volume indicated in the colorimetric procedure for the parameter under analysis.
9. Continue with the colorimetric procedure.

4.2.2.2 For the total Kjeldahl Nitrogen colorimetric method

Consult the spectrophotometer or colorimeter procedure to complete the TKN analysis. The following is only a guide to use if a procedure is not available.

1. Pipet an appropriate analysis volume into a mixing graduated cylinder.
2. Add one drop of TKN Indicator (Cat. No. 2251900).
3. Add one drop of 8 N KOH Standard Solution (Catalog No. 28232H), swirling between each addition, until the first flash of pale blue appears (pH 3).
4. Add one drop of 1 N KOH (Cat. No. 2314426). Stopper the cylinder and invert several times to mix.

   Note: *View the cylinder from the top against a white background. Compare the cylinder to a second cylinder filled to the same volume with deionized water.*

5. Continue to add 1 N KOH in this manner until the first permanent blue color appears.
6. Add deionized water to the volume indicated in the colorimetric procedure for the parameter under analysis.

Continue with the colorimetric procedure.

This section provides guidelines for laboratory waste management. These guidelines are only a summary of basic USEPA requirements and do not relieve the user from complying with the complete regulations contained in the Code of Federal Regulations (CFR). The regulations may change or additional state and local laws may apply; waste generators are responsible for knowing and following all the laws and regulations that apply to their operations.

5.1 Waste minimization

The most effective way to decrease waste management problems and expense is through waste minimization. To do this:

- Use the smallest sample size that will produce accurate results.
- Where possible, choose methods that use reagents that pose fewer hazards.
- Eliminate the need to dispose of out-dated materials by purchasing in smaller quantities.
- Use biodegradable detergents to clean glassware and apparatus unless solvents or acids are specifically required.

5.2 Regulatory overview

The Resource Conservation and Recovery Act (RCRA) controls all solid waste disposal with an emphasis on hazardous waste. Title 40 Code of Federal Regulations (CFR) part 260 contains the federal hazardous waste disposal regulations issued in accordance with the RCRA. The regulations create a system to identify hazardous wastes and track waste generation, transport and ultimate disposal from cradle to grave. Each facility involved in hazardous waste management must be registered with the USEPA, with the exception of conditionally exempt small quantity generators.

Federal regulations recognize three categories of generators with those generating larger amounts of waste being under stricter control.

The categories are:

Conditionally Exempt Small Quantity Generator— less than 100 kg (220 lb) per month

Small Quantity Generator— between 100 kg (220 lb) and 1000 kg (2200 lb) per month

Large Quantity Generator— greater than 1000 kg (2200 lb) per month.

5.3 Hazardous waste

5.3.1 Definition

For regulatory purposes, a *hazardous waste* is a material that is subject to special consideration by the USEPA under 40 CFR 261. State or local authorities may also designate additional materials as hazardous waste in their areas.

Many toxic compounds are not regulated, but improper management or disposal may lead to legal problems under CERCLA (Superfund) or common law tort.

The definition given by 40 CFR 261 defines a hazardous waste as a solid waste that is not excluded from regulation and meets one or more of the following criteria:

- It is a discarded commercial chemical product, off-specification species, container residue or spill residue of materials specifically listed in 40 CFR 261.33 (P- and U-codes);
- It is a waste from a specific source listed in 40 CFR 261.32 (K-code);
- It is a waste from a non-specific source listed in 40 CFR 261.31 (F-code);
- and/or
- It displays any of the following characteristics of hazardous waste:
  - ignitability
  - corrosivity
  - reactivity
  - toxicity

There are exceptions to these regulations; review the regulations to determine applicable exclusions.

5.3.2 Sample codes

Hazardous wastes are managed by specific codes assigned in 40 CFR 261.20–261.33. These codes are provided to help identify hazardous waste. The generator is responsible for making the actual waste code determination.

Selected characteristic waste codes for chemicals which may be generated using methods for water analysis are given in the following table. A complete list of waste codes is found in 40 CFR 261.20 through 40 CFR 261.33.

Table 14 Hazardous waste codes

Characteristic	USEPA code	Chemical abstract services (CAS) No.	Regulatory level (mg/L)
Corrosivity	D002	na	na
Ignitability	D001	na	na
Reactivity	D003	na	na
Arsenic	D004	6440-38-2	5.0
Barium	D005	6440-39-3	100.0
Benzene	D018	71-43-2	0.5
Cadmium	D006	7440-43-9	1.0
Chloroform	D022	67-66-3	6.0
Chromium	D007	7440-47-3	5.0
Lead	D008	7439-92-1	5.0
Mercury	D009	7439-97-6	0.2
Selenium	D010	7782-49-2	1.0
Silver	D011	7440-22-4	5.0

5.3.3 How to determine if waste is hazardous

Federal laws do not require testing of material to decide if it is a hazardous waste. If the product is not specifically listed in the regulations, apply product or generator knowledge to decide if it is hazardous. Often, there is enough information on a Material Safety Data Sheet (MSDS) to decide. Look for characteristics of a hazardous waste:

- Flash point is below 60 °C (140 °F) or it is classified by DOT as an oxidizer (D001).
- The pH of the material is £2 orŠ³12.5 (D002).
- The material is unstable, reacts violently with water, may generate toxic gases when mixed with water (D003).
- It is toxic (D004–D043).

Use the chemical composition data to decide if a material is toxic based on the concentration of certain contaminants (Heavy metals and a number of organic compounds). If the waste is a liquid, compare the concentration of contaminants to the concentrations listed in 40 CFR 26. If the waste is a solid, analyze the sample by the Toxicity Characteristic Leachability Procedure (TCLP) and then compare the results to the concentrations in 40 CFR 261.24. Levels above the threshold amounts are considered hazardous.

For more information on using the MSDS, see *Material safety data sheets*.

Some tests use or produce a number of chemicals that make the end product a hazardous waste; for example, the COD tests and Nessler reagent. Hazardous waste status may also result from substances present in the sample.

5.3.4 Disposal

Hazardous waste must be managed and disposed of according to federal, state and local regulations. The waste generator is responsible for making hazardous waste determinations. Analysts should check with their facility's environmental compliance department for specific instructions.

Most hazardous wastes should be handled by treatment, storage and disposal facilities (TSDF) that have USEPA permits. In some cases, the generator may treat the hazardous waste, but may need a permit from the USEPA and/or state agency. Laboratories are not exempt from these regulations. If the facility is a "Conditionally Exempt Small Quantity Generator," special rules may apply. Check 40 CFR 261 to determine the laws and rules that apply for a given generator.

The most common allowed treatment is elementary neutralization. This applies to wastes that are hazardous only because they are corrosive or are listed only for that reason. Neutralize acidic solutions by adding a base such as sodium hydroxide; neutralize basic solutions by adding an acid such as hydrochloric acid. Slowly add the neutralizing agent while stirring. Monitor the pH. When it is at or near 7, the material is neutralized and may be flushed down the drain. Many generated wastes may be treated in this manner.

Other chemical or physical treatments such as cyanide destruction or evaporation may require a permit. Check with the environmental department or local regulators to determine which rules apply to each facility.

Laboratory chemicals may be mixed and disposed of with other hazardous wastes generated at a facility. They may also be accumulated in accordance with 40 CFR 262.34 satellite accumulation rules. After collection they may be disposed of in a *labpack*. Many environmental and hazardous waste companies offer labpacking services. They will inventory, sort, pack and arrange for proper disposal of hazardous waste. Find companies offering these services in the Yellow Pages under "Waste Disposal — Hazardous" or contact state and local regulators for assistance.

5.4 Management of specific wastes

Recyling programs for some forms of laboratory waste are available through Hach Company. Obtain information on recycling services by calling 1-800-227-4224 or by visiting www.hach.com."

Several documents are also available to assist in managing generated waste. Obtain available documents by calling 1-800-227-4224 or 970-669-3050 and requesting the literature codes given:

Table 15 Waste management literature

Literature code	Title
9323	Mercury Waste Disposal Firms
9324	RCRA Waste Disposal Information
9325	COD Waste Disposal Information
9326	COD Heavy Metal Concentrations

5.4.1 Special considerations for Cyanide-containing materials

Several procedures in this manual use reagents that contain cyanide compounds. These materials are regulated as reactive waste (D003) by the Federal RCRA. Instructions provided with each procedure explain how to collect these materials for proper disposal. It is imperative that these materials be handled safely to prevent the release of hydrogen cyanide gas (an extremely toxic material with the smell of bitter almonds). Most cyanide compounds are stable and can be safely stored for disposal, in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes that may contain lower pH materials such as acids or even water.

If a cyanide-containing compound is spilled, avoid exposure to hydrogen cyanide gas. Take the following steps to destroy the cyanide compounds in an emergency:

1. Use a fume hood, supplied air or self-contained breathing apparatus.
2. While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and either calcium hypochlorite or sodium hypochlorite (household bleach).
3. Add an excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.
4. Neutralize the solution and flush it down the drain with a large amount of water. If the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Never flush untreated hazardous wastes down the drain.

5.5 Resources

Many sources of information on proper waste management are available. The USEPA has a hotline number for questions about the Resource Conservation and Recovery Act (RCRA). The RCRA Hotline number is 1-800-424-9346. Copies of the appropriate regulations are available. Federal hazardous waste regulations are found in 40 CFR 260-99. Obtain this book from the U.S. Government Printing Office or an alternate vendor. Other documents that may be helpful to the hazardous waste manager in the laboratory include:

- Task Force on Laboratory Waste Management. *Laboratory Waste Management, A Guidebook*; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1994.
- Task Force on Laboratory Waste Management. *Waste Management Manual for Laboratory Personnel*; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1990.

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- Armour, Margaret-Ann. *Hazardous Laboratory Chemicals Disposal Guide*; CRC Press: Boca Raton, FL, 1991.
- *Environmental Health and Safety Manager's Handbook*; Government Institutes, Inc.: Rockville, MD, 1988.
- Lunn, G.; Sansone, E.B. *Destruction of Hazardous Chemicals in the Laboratory*; John Wiley and Sons: New York, 1990.
- National Research Council. *Prudent Practices for Disposal of Chemicals from Laboratories*; National Academy Press: Washington, DC, 1983.
- National Research Council. *Prudent Practices for Handling Hazardous Chemicals in Laboratories*; National Academy Press: Washington, DC, 1981.
- Environmental Protection Agency, Office of Solid Waste and Emergency Response. *The RCRA Orientation Manual*; U.S. Government Printing Office: Washington, DC, 1991.
- Environmental Protection Agency, Office of Solid Waste and Emergency Response. *Understanding the Small Quantity Generator Hazardous Waste Rules: A Handbook for Small Business*; U.S. Government Printing Office: Washington, DC, 1986.

5.6 Safety

Safety is the responsibility of every analyst. Many chemical procedures use potentially hazardous chemicals and equipment; it is important to prevent accidents by practicing good laboratory techniques. The following guidelines apply to water analysis and are not intended to cover every aspect of safety.

5.6.1 Read labels carefully

Read each reagent label carefully. Pay particular attention to the precautions given. Never remove or cover the label on a container while it contains reagent. Do not put a different reagent into a labeled container without changing the label. When preparing a reagent or standard solution, label the container clearly. If a label is hard to read, relabel promptly according to the hazard communication program.

Warning labels also appear on some of the apparatus used with the test procedures. The protective shields with the Digesdahl Digestion Apparatus point out potential hazards. Be sure these shields are in place during use and observe the precautions on the label.

5.6.2 Protective equipment

Use the right protective equipment for the chemicals and procedures. The MSDS contains this information. Protective equipment may include:

- Eye protection, such as safety glasses or goggles, to protect from flying objects or chemical splashes.
- Gloves to protect skin from toxic or corrosive materials, sharp objects, very hot or very cold materials or broken glass. Use tongs or finger cots when transferring hot apparatus.
- Laboratory coats or splash aprons to protect skin and clothing from splashes.
- Footwear to protect feet from spills. Open toed shoes should not be worn in chemistry settings.

- Respirators may be needed if adequate ventilation, such as fume hoods, are not available. Use fume hoods when directed to do so by the procedure or as recommended in the MSDS. For many procedures, adequate ventilation is enough if there is plenty of fresh air and air exhaust to protect against unnecessary exposure to chemicals.

5.6.3 First aid equipment and supplies

Most first aid instructions for chemical splashes in eyes or on skin call for thorough flushing with water. Laboratories should have eyewash and shower stations. For field work, carry a portable eyewash unit. Laboratories should also have appropriate fire extinguishers and fume hoods.

5.6.4 General safety rules

Follow these rules when working with toxic and hazardous chemicals:

- **Never** pipet by mouth. Always use a mechanical pipet or pipet bulb to avoid ingesting chemicals.
- Follow test procedures carefully and observe all precautionary measures. Read the entire procedure before beginning.
- Wipe up all spills promptly. Get proper training and have the right response equipment to clean up spills. See the safety director for more information.
- **Do not** smoke, eat or drink in an area where toxic or irritating chemicals are used.
- Use reagents and equipment only as directed in the test procedure.
- **Do not** use damaged labware and broken equipment.
- Minimize all chemical exposures. **Do not** breathe vapors or let chemicals touch the skin. Wash hands after using chemicals.
- Keep work areas **neat** and **clean**.
- **Do not** block exits or access to emergency equipment.

5.7 Material safety data sheets

Material safety data sheets (MSDS) describe the hazards of chemical products. This section explains the information found on the MSDS and tells how to locate important information for safety and waste disposal. The information provided on the MSDS applies to the product as sold by a specific manufacturer. The properties of any mixtures obtained by using this product will be different.

5.7.1 How to obtain an MSDS

The MSDS is shipped to a customer with the first order of any chemical product. A new MSDS may be sent when the information on the data sheet is updated. Please review all new MSDS for new information. To obtain another copy of an MSDS, call 1-800-227-4224 or download directly from www.hach.com/msdsinfo.htm.

5.7.2 Sections of an MSDS

Each MSDS has ten sections. The sections and the information included are described below.

Header information
The manufacturer order number, MSDS date, change number, company address and telephone number and emergency telephone numbers are listed at the top of the MSDS.

5.7.2.1 Product identification

This section contains:

- Product name
- Chemical Abstract Services (CAS) number
- Chemical name
- Chemical formula, if appropriate
- Chemical family to which the material belongs

5.7.2.2 Ingredients

This section lists each component in the product. It contains the following information for each component:·

- **PCT:** Percent by weight of this component
- **CAS NO.:** Chemical Abstract Services (CAS) registry number for this component
- **SARA:** Superfund Amendments and Reauthorization Act, better known as the "Community Right to Know Law", informs if the component is listed in SARA 313. If the component is listed and the facility uses more than the specified amount, report use to the USEPA every year.
- **TLV:** Threshold Limit Value. The maximum airborne concentration for an 8-hour exposure that is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).
- **PEL:** Permissible Exposure Limit. The maximum airborne concentration for an 8-hour exposure that is regulated by the Occupational Safety and Health Administration (OSHA).
- **HAZARD:** Physical and health hazards of the component are explained.

5.7.2.3 Physical data

The physical properties of the product are given in this section. They include the physical state, color, odor, solubility, boiling point, melting point, specific gravity, pH, vapor density, evaporation rate, corrosivity, stability and storage precautions.

5.7.2.4 Fire, explosion hazard and reactivity data

This section contains the flash point and flammable limits of the material. It also includes how to fight fires if the material catches on fire. Key terms in this section include:

- Flash point: The temperature at which a liquid will give off enough flammable vapor to ignite.
- Flammability and ignitability are usually defined by the flash point.
- Lower Flammable Limit (LFL or LEL): The lowest concentration that will produce a fire or flash when an ignition source is present.
- Upper Flammable Limit (UFL or UEL): The vapor concentration in air above which the concentration is too rich to burn.
- NFPA Codes: The National Fire Protection Association (NFPA) has a system to rate the degree of hazards presented by a chemical. These codes are usually placed in a colored diamond. The codes range from 0 for minimal hazard to 4 for extreme hazard. They are grouped into the following hazards: health (blue), flammability (red), reactivity (yellow) and special hazards (white).

5.7.2.5 Health hazard data

This section describes the pathways by which the chemical can enter the body (i.e., ingestion, inhalation, skin contact). It also gives acute (immediate) and chronic (long-term) health effects. If the material causes cancer or genetic damage, it is stated in this section.

5.7.2.6 Precautionary measures

This section contains special precautions for the material. These may include special storage instructions, handling instructions, conditions to avoid and protective equipment required to use this material safely.

5.7.2.7 First aid

First aid instructions for exposures to the chemical are given in this section. Be sure to read this section before inducing vomiting in a victim. Some chemicals are better treated by not inducing vomiting. Seek prompt medical attention for all chemical exposures.

5.7.2.8 Spill and disposal procedures

This section explains safe practices for cleaning up and disposing of spilled material. For more information, see *Hazardous waste*. **The waste generator is ultimately responsible for meeting the federal, state and local laws that apply to each facility.**

5.7.2.9 Transportation data

Domestic and International shipping information is provided in this section. It gives shipping name, hazard class and ID number of the product.

5.7.2.10 References

This section lists the reference materials used to write the MSDS.

Following the Reference section, the product will be listed as having SARA 313 chemicals or California Proposition 65 List Chemicals, if applicable. Also found here is any special information about the product.

5.7.3 OSHA chemical hygiene plan

The Occupational Safety and Health Administration (OSHA) enforces laws controlling exposure to hazardous chemicals in laboratories. These regulations are in Title 29 CFR 1910.1450. The regulations apply to all employers who use hazardous chemicals and require employers to develop and use a written Chemical Hygiene Plan and to appoint a qualified person as the Chemical Hygiene Officer.

Section 6 International Guideline Comparison

The following table shows a comparison between international drinking water and FDA bottled water guidelines:

Table 16 Comparison of international drinking water and FDA bottled water guidelines[1]

Parameter	USEPA[2] maximum contaminant level (MCL)	Canada[3] maximum acceptable concentration	EEC[4] maximum admissible concentration	Japan[5] maximum admissible concentration	WHO[6] guideline	Bottled Water U.S. Federal Drug Administration level
Aluminum	0.05–0.2 mg/L[7]	—	0.2 mg/L	0.2 mg/L	0.2 mg/L	—
Ammonium	—	—	0.5 mg/L	No standard	1.5 mg/L	—
Antimony	0.006 mg/L	—	0.01 mg/L	0.002 mg/L[8]	0.005 mg/L	—
Arsenic	0.05 mg/L	0.025 mg/L	0.05 mg/L	0.01 mg/L	0.01 mg/L	0.05 mg/L
Barium	2.0 mg/L	1.0 mg/L	No standard	No standard	0.7 mg/L	2.0 mg/L
Boron	—	5.0 mg/L	1.0 mg/L	0.2 mg/L[8]	0.3 mg/L	—
Cadmium	0.005 mg/L	0.005 mg/L	0.005 mg/L	0.01 mg/L	0.003 mg/L	0.005 mg/L
Chloride	250 mg/L[7]	250 mg/L	250 mg/L	200 mg/L	250 mg/L	
Chromium	0.1 mg/L	0.05 mg/L	0.05 mg/L	0.05 mg/L	0.05 mg/L	0.1 mg/L
Coliforms, total Organisms/100 mL	£% positive	0	0 or MPN £1	0	0	£1 MF
Coliforms (*E. coli*) Organisms/100 mL	0	0	0	0	0	—
Color	15 cu[7]	15 cu	20 mg Pt-Co/L	5 cu	15 cu	<15 cu
Copper	1.3 mg/L[7]	1.0 mg/L	2.0 mg/L	1.0 mg/L	1–2 mg/L	1.0 mg/L
Cyanides	0.2 mg/L	0.2 mg/L	0.05 mg/L	0.01 mg/L	0.07 mg/L	—
Fluoride	2.0-4.0 mg/L[7]	1.5 mg/L	0.7-1.5 mg/L	0.8 mg/L	1.5 mg/L	—
Hardness	—	—	50 mg/L	300 mg/L	—	—
Iron	0.3 mg/L[7]	0.3 mg/L	0.2 mg/L	0.3 mg/L	0.3 mg/L	—
Lead	0.015 mg/L	0.01 mg/L	0.01 mg/L	0.05 mg/L	0.01 mg/L	0.005 mg/L
Manganese	0.05 mg/L	0.05 mg/L	0.05 mg/L	0.01–0.05 mg/L	0.1–0.5 mg/L	—
Mercury	0.002 mg/L	0.001 mg/L	0.001 mg/L	0.0005 mg/L	0.001 mg/L	0.002 mg/L
Molybdenum	—	—	—	0.07 mg/L	0.07 mg/L	—
Nickel	0.1 mg/L	—	0.02 mg/L	0.01 mg/L[8]	0.02 mg/L	—
Nitrate/Nitrite, total	10.0 mg/L as N	—	—	10.0 mg/L as N	—	10 mg/L as N
Nitrates	10.0 mg/L as N	10.0 mg/L as N	50 mg/L	10 mg/L as N	50 mg/L as NO_3^-	—
Nitrites	1 mg/L as N	3.2 mg/L	0.1 mg/L	10 mg/L	3 mg/L as NO_2^-	1 mg/L as N
Odor	3 TON[9]	—	2 dilution no. @ 12 °C; 3 dilution no. @ 25 °C.	3 TON	—	—
pH	6.5–8.5	6.5–8.5	6.5–9.5	5.8–8.6	6.5–8.5	—
Phosphorus	—	—	5 mg/L	No Standard	—	—
Phenols	—	0.002 mg/L	0.5 µg/L C_6H_5OH	0.005 mg/L	—	—

Table 16 Comparison of international drinking water and FDA bottled water guidelines[1] (continued)

Parameter	USEPA[2] maximum contaminant level (MCL)	Canada[3] maximum acceptable concentration	EEC[4] maximum admissible concentration	Japan[5] maximum admissible concentration	WHO[6] guideline	Bottled Water U.S. Federal Drug Administration level
Potassium	—	—	12 mg/L	No Standard	—	—
Selenium	0.05 mg/L	0.01 mg/L	0.01 mg/L	0.01 mg/L	0.01 mg/L	0.05 mg/L
Silica Dioxide	—	—	10 mg/L	No Standard	—	—
Silver	0.1 mg/L[7]	0.05 mg/L	0.01 mg/L	No standard	No standard	—
Solids, total dissolved	500 mg/L[7]	500 mg/L	No standard	500 mg/L	1000 mg/L	—
Sodium	—	—	75-150 mg/L	200 mg/L	200 mg/L	—
Sulfate	250 mg/L[7]	500 mg/L	250 mg/L	No Standard	250 mg/L	—
Turbidity	0.5-5 NTU	1 NTU	4 JTU	1–2 units	5 NTU	<5 NTU
Zinc	5 mg/L[7]	5.0 mg/L	No Standard	1.0 mg/L	3.0 mg/L	—

[1] Contact the local regulatory agency for the most current information.

[2] United States Environmental Protection Agency.

[3] These limits are established by Health Canada.

[4] In the EEC (European Economic Community), limits are set by the European Committee for Environmental Legislation.

[5] In Japan, limits are established by the Ministry of Health and Welfare.

[6] World Health Organization.

[7] U.S. Secondary MCL.

[8] Identified as a parameter to be regulated in the future.

[9] Threshold Odor Number.

Section 7 Definitions of USEPA Approved and Accepted

7.1 USEPA approved

The United States Environmental Protection Agency (USEPA) establishes limits for maximum contamination levels of certain constituents in water. It also requires that specific methodology be used to analyze for these constituents. Sometimes the USEPA develops these methods; more often, the USEPA evaluates methods developed by manufacturers, professional groups, and public agencies such as:

- American Public Health Association
- American Water Works Association
- Water Environmental Federation
- American Society for Testing and Materials
- United States Geological Survey
- Association of Official Analytical Chemists

When a method meets the USEPA criteria, it is *approved*. All USEPA approved methods are cited in the *Federal Register* and compiled in the *Code of Federal Regulations*. USEPA-approved methods may be used for reporting results to the USEPA and other regulatory agencies.

7.2 USEPA accepted

Some procedures are ***equivalent*** to USEPA approved methods. Even though minor modifications exist, the USEPA has reviewed and accepted certain procedures for reporting purposes. These methods are not published in the *Federal Register,* but are referenced to the equivalent USEPA method in the procedure.

Sample cells and apparatus

Sample cells

2495402

2401906

1353702

1993500

2095000

2095100

2410212

2410222

2427606

4822800

2629250

5940506

2612602

4864302

2122800

Instruments and adapters

DR 5000

DR 2800 and DR 2700

DR2500

DR2400

LZV583

LZV584

LZV585

LZV646

LZV479

5940400

5912200

HACH

Acid Determination and Base Determination

Method 8200 and 8233

1 to 4000 meq/L

Digital Titrator

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

Four drops of Phenolphthalein Indicator Solution[1] can be substituted for the Phenolphthalein Indicator Powder Pillow.
A pH meter can be used in place of the indicators. The end point for the titration is pH 8.3.
To change the result from meq/L to normality (N), divide the result in meq/L by 1000.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

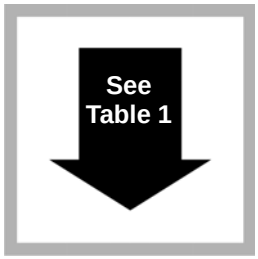
[1] See *Optional reagents and apparatus*.

Collect the following items:

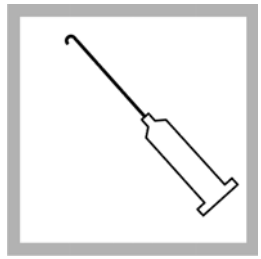
Description	Quantity
Phenolphthalein Indicator Powder Pillow	1 pillow
Ttitration cartridge (see *Range-specific information*)	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Acid determination (Method 8200)

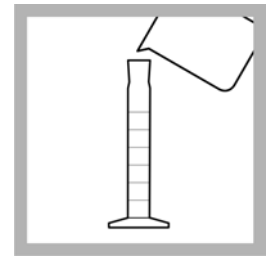


1. Select a sample volume and sodium hydroxide titration cartridge from the *Range-specific information* table.



2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

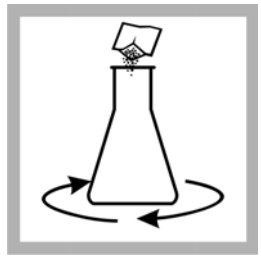
3. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.



4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table.

Acid determination (Method 8200)

5. Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.



6. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix. The solution should be colorless.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until a light pink color forms and persists for 30 seconds.

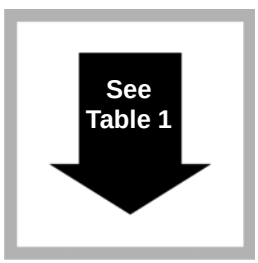
Write down the number of digits displayed on the counter.

8. Use the multiplier in the *Range-specific information* table to calculate the concentration:

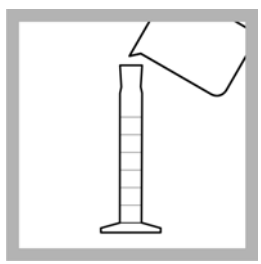
digits x multiplier = meq/L acid

Example: 100 mL of sample was titrated with the 8.00 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 meq/L acid

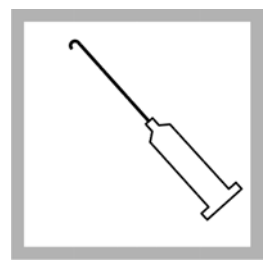
Base determination (Method 8233)



1. Select a sample volume and acid titration cartridge from the *Range-specific information* table.



2. Use a graduated cylinder or pipet to measure the sample volume from the*Range-specific information* table.

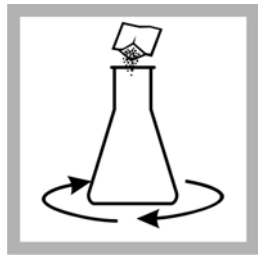


3. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

4. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.

Base determination (Method 8233)

5. Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.



6. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix. The solution should be pink.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the solution is colorless.

Write down the number of digits displayed on the counter.

8. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = meq/L base

Example: 100 mL of sample was titrated with the 8.00 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 meq/L base

Table 17 Range-specific information

Range (meq/L)	Sample volume (mL)	Titration cartridge[1] (N)	Multiplier
1–4	100	1.600	0.02
4–10	50	1.600	0.04
10–40	100	8.00	0.1
20–80	50	8.00	0.2
50–200	20	8.00	0.5
100–400	10	8.00	1.0
200–800	5	8.00	2.0
500–2000	2	8.00	5.0
1000–4000	1	8.00	10.0

[1] For acid determinations, use a sodium hydroxide titration cartridge. For base determinations, use a hydrochloric acid or sulfuric acid cartridge.

Interferences

Color or turbidity can mask the color change of the end point. Use a pH meter instead of the color indicators and titrate to a pH of 8.3 to duplicate the phenolphthalein end point.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Fill completely and cap tightly.
- Prevent excessive agitation or prolonged exposure to air.
- Complete the test procedure as soon as possible after collection for best accuracy. The sample can be stored for at least 24 hours if cooled to 4 °C (39 °F) or below.
- Warm to room temperature before the test is started.

Accuracy check

The standard solution method can be used to confirm analytical technique and reagent performance.

Standard solution method
Complete the following test to make sure the concentration of the titrant is accurate.

Required for accuracy check:

- For acid determination—0.500 N Sulfuric Acid Standard Solution
- For base determination—Alkalinity Standard Solution, 0.500 N Na_2CO_3

1. Add the standard solution to an Erlenmeyer flask:
   - If using the 1.600 N Titration Cartridge, pipet 1.0 mL of the standard solution into the Erlenmeyer flask and dilute to approximately 100 mL with deionized water.
   - If using the 8.00 N Titration Cartridge, pipet 5.0 mL of the standard solution into the Erlenmeyer flask and dilute to approximately 100 mL with deionized water.
2. Add one Phenolphthalein Powder Pillow and swirl to mix.
3. Follow the test procedure to titrate the standard to the end point. The expected digits to reach the end point are 250.

Summary of method

A measured amount of sample is treated with a phenolphthalein colorimetric indicator and then titrated with a strong acid or base to a pH of 8.3. The amount of titrant used is directly proportional to the milliequivalents of acid or base in the sample. These titrations also can be performed using a pH meter instead of a colorimetric indicator.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hydrochloric Acid Titration Cartridge, 8.00 N	varies	each	1439001
Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
Sodium Hydroxide Titration Cartridge, 1.600 N	varies	each	1437901
Sodium Hydroxide Titration Cartridge, 8.00 N	varies	each	1438101
Sulfuric Acid Titration Cartridge, 1.600 N	varies	each	1438901
Sulfuric Acid Titration Cartridge, 8.00 N	varies	each	1439101

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator		each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 5-mL	1	each	50837
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842

Recommended standards

Description	Unit	Catalog number
Alkalinity Standard Solution, 0.500 N Na_2CO_3, 10-mL ampules	16/pkg	1427810
Sulfuric Acid Standard Solution, 0.500 N	100 mL	212132

Optional reagents and apparatus

Description	Unit	Catalog number
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Water, deionized	500 mL	27249
Pipet, volumetric, Class A, 1 mL	each	1451535
Pipet, volumetric, Class A, 2 mL	each	1451536
Pipet, volumetric, Class A, 5 mL	each	1451537
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet, volumetric, Class A, 20 mL	each	1451520
Pipet Filler, safety bulb	each	1465100
Bottle, sampling, poly, 500 mL	each	2087079
pH meter	each	—
Delivery Tube, 180° hook	5/pkg	1720500
TitraStir stir plate, 115 Vac	each	1940000
TitraStir stir plate, 230 Vac	each	1940010
Stir Bar, Octagonal 28.6 mm x 7.9 mm	each	2095352

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Edition 7

Acid-Base

DOC316.53.01148

Sodium Hydroxide for meq/L of Acid — Method 8288
Sulfuric Acid for meq/L of Base — Method 8289

(0 to 25,000 meq/L) — **Buret Titration**

Scope and Application: For water, wastewater and seawater

Test preparation

Before starting the test:

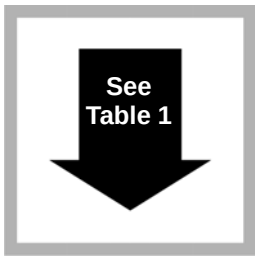
Read the entire procedure before starting the test.
Four drops of Phenolphthalein Indicator Solution can be substituted for the Phenolphthalein Indicator Powder Pillow.
To determine the normality of the sample, divide the milliequivalents per liter obtained by 1000.

Collect the following items:

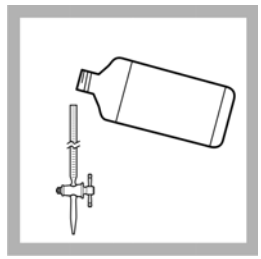
Description	Quantity
Phenolphthalein indicator powder pillow	1
Sodium hydroxide standard solution (acid test)	varies[1]
Sulfuric acid standard solution (base test)	varies[1]
Buret clamp	1
Buret, Class A	1
Graduated cylinder	varies[1]
Erlenmeyer flask	1
Support Stand	1

[1] See *Consumables and replacement items*.

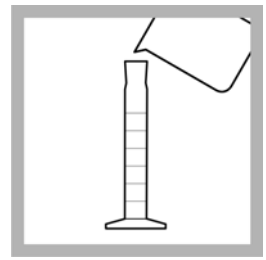
Sodium hydroxide acid determination (Method 8288)



1. Select the sample volume that corresponds to the expected acid concentration in milliequivalents per liter (meq/L) or normality (N) from the *Sample volume selection for expected acid concentration* table.



2. Fill a 25-mL buret to the zero mark with the appropriate Sodium Hydroxide Solution.



3. Measure the sample volume from the *Sample volume selection for expected acid concentration* table using a graduated cylinder or pipet.

4. Pour the sample into a 250-mL Erlenmeyer flask. Dilute to 50–100 mL with deionized water, if necessary.

5. Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix. The solution should be colorless.

6. Swirl the flask while titrating until a light pink color forms and persists for 30 seconds.

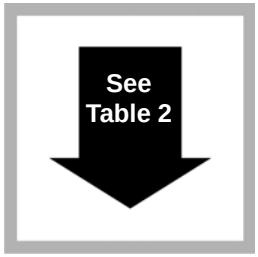
7. Calculate:

mL NaOH Titrant × Multiplier used = meq/L of Acid

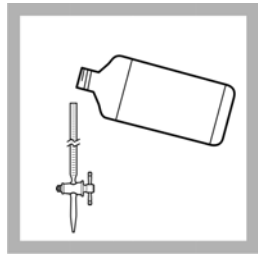
Table 18 Sample volume selection for expected acid concentration

Range		Sample Volume (mL)	Sodium Hydroxide Standard Solution Titrant (N)	Catalog Number	Multiplier
(meq/L)	(N)				
0–10	0–0.010	50	0.020	19353	0.4
10–100	0.010–0.100	25	0.100	19153	4
100–500	0.100–0.500	50	1.000	104553	20
500–2500	0.500–2.50	10	1.000	104553	100
2500–25000	2.50–25.0	1	1.000	104553	1000

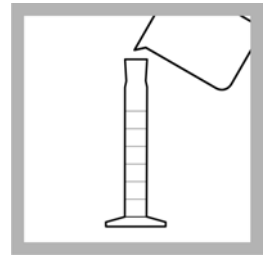
Sulfuric acid base determination (Method 8289)



1. Select the sample volume that corresponds to the expected base concentration in milliequivalents per liter (meq/L) or normality (N) from the *Sample volume selection for expected base concentration* table.



2. Fill a 25-mL buret to the zero mark with the appropriate Sulfuric Acid Standard Solution.



3. Measure the sample volume from Table 2 using a graduated cylinder or pipet.

4. Pour the sample into a 250-mL Erlenmeyer flask. Dilute to 50–100 mL with deionized water, if necessary.

5. Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix. The solution should turn pink.

6. Swirl the flask while titrating just until the solution turns colorless.

7. Calculate:

mL Sulfuric Acid Titrant X Multiplier used = meq/L of Base

Table 19 Sample volume selection for expected base concentration

Range		Sample Volume (mL)	Sulfuric Acid Standard Solution Titrant (N)	Catalog Number	Multiplier
(meq/L)	(N)				
0–10	0–0.010	50	0.020	20353	0.4
10–100	0.010–0.100	25	0.100	20253	4
100–500	0.100–0.500	50	1.000	127053	20
500–2500	0.500–2.50	10	1.000	127053	100
2500–25000	2.50–25.0	1	1.000	127053	1000

Interferences

Highly colored or turbid samples may mask the color change at the end point. Use a pH meter for these samples. Titrate to pH 8.3 to duplicate the colorimetric phenolphthalein end point.

Sampling and storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Minimize agitation or prolonged exposure to air. Samples may be stored at least 24 hours by cooling to 4 °C (39 °F) or below if they cannot be analyzed immediately. Warm to room temperature before analyzing.

Accuracy check

For acid determinations, use a 20-mL volumetric pipet to measure Sulfuric Acid Standard Solution of the same normality as the Sodium Hydroxide Standard Solution being used. Pipet the sulfuric acid into an Erlenmeyer flask. Dilute to the 100-mL mark with deionized water. Perform the test; 20 mL of sodium hydroxide should be required.

For base determinations, use a 20-mL volumetric pipet to measure Sodium Hydroxide Standard Solution of the same normality as the Sulfuric Acid Standard Solution being used. Pipet sodium hydroxide into an Erlenmeyer flask. Dilute to the 100-mL mark with deionized water. Perform the test; 20 mL of sulfuric acid should be required.

If incorrect results are obtained, refer to Method Performance in the Water Analysis Guide.

Summary of method

A measured amount of sample is treated with a phenolphthalein colorimetric indicator and then titrated with a strong acid or base to pH 8.3. The amount of titrant used is directly proportional to the milliequivalents of acid or base in the sample. These titrations also can be performed using a pH meter instead of a colorimetric indicator.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Catalog number
Phenolphthalein Indicator Powder Pillow	1 pillow	100/pkg	94299
Water, deionized	varies	4 L	27256
For acid, pick one or more based on range:			
Sodium Hydroxide Standard Solution, 0.020 N	—	1 L	19353
Sodium Hydroxide Standard Solution, 0.100 N	—	1 L	19153
Sodium Hydroxide Standard Solution, 1.000 N	—	1 L	104553
For base, pick one or more based on range:			
Sulfuric Acid Standard Solution, 0.020 N	—	1 L	20353
Sulfuric Acid Standard Solution, 0.100 N	—	1 L	20253
Sulfuric Acid Standard Solution, 1.000 N	—	1 L	127053

Required apparatus

Description	Quantity/test	Unit	Catalog number
Buret clamp, double	1	each	32800
Buret, Class A, 25-mL	1	each	2636540
Pick one or more based on sample volume:			
Cylinder, graduated, 5-mL	—	each	50837
Cylinder, graduated, 10-mL	—	each	50838
Cylinder, graduated, 25-mL	—	each	50840
Cylinder, graduated, 50-mL	—	each	50841
Flask, Erlenmeyer, 250-mL	1	each	50546
Pipet, volumetric, Class A, 1-mL	1	each	1451535
Pipet, volumetric, Class A, 10-mL	1	each	1451538
Support stand	1	each	56300

Optional items

Description	Unit	Catalog number
Pipet, 20-mL, volumetric	each	1451520
Pipet filler, Safety bulb	each	1465100
Phenolphthalein Indicator Solution, 5 g/L100 mL	—	16232

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Edition 7

Acidity, Methyl Orange

DOC316.53.01150

Sodium Hydroxide with a Buret[1]

Method 8219

(0 to 10,000 mg/L as $CaCO_3$)

Buret Titration

Scope and Application: For water, wastewater and seawater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

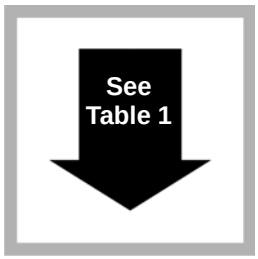
Read the entire procedure before starting the test.
Avoid excessive agitation of the sample to prevent the loss of dissolved gases such as carbon dioxide, hydrogen sulfide or ammonia.
Six drops of Phenolphthalein Indicator Solution may be substituted for the Phenolphthalein Powder Pillow.
When determining Methyl Orange Acidity, six drops of Bromphenol Blue Indicator Solution can be substituted for the Bromphenol Blue Indicator Powder Pillow. Methyl Orange Indicator Solution can also be substituted for the indicator. When using the Methyl Orange Indicator Solution, the end point color change then becomes red to orange.
The Methyl Orange and Phenolphthalein Acidity procedures can be run sequentially if both values are desired. First, titrate to pH 3.7 and record the amount of sodium hydroxide used. Then titrate to pH 8.3.
Phenolphthalein acidity in mg/L $CaCO_3$ x 0.0584 = grains per gallon

Collect the following items:

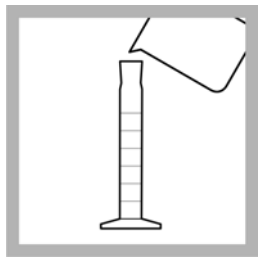
Description	Quantity
Bromphenol Blue indicator powder pillow	1
Sodium hydroxide standard solution, 0.020 N	varies[1]
Buret clamp	1
Buret, Teflon plug, Class A	1
Graduated cylinder	varies[1]
Erlenmeyer flask	1
Funnel, Micro	1
Support Stand	1

[1] See *Consumables and replacement items* on page 86.

Buret titration (Method 8219)



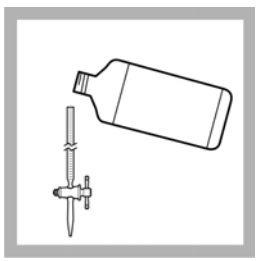
1. Select a sample volume from the *Sample volume selection for expected concentration* table that corresponds to the expected acidity concentration in mg/L as calcium carbonate ($CaCO_3$).



2. Use a graduated cylinder or pipet to measure the sample volume.

3. Transfer the sample into a 250-mL Erlenmeyer flask. Dilute to about 50-mL with deionized water if necessary.

4. Add the contents of one Bromphenol Blue Indicator Powder Pillow. Swirl to mix. (Omit this step when using a pH meter.)



5. Fill a 50-mL buret to the zero mark with 0.020 N Sodium Hydroxide Standard Solution.

6. While swirling the flask, titrate the sample with 0.020 N Sodium Hydroxide Standard Solution until the solution color changes from yellow to pure green (pH 3.7).

7. Calculate:

mL Titrant × multiplier used = mg/L methyl orange acidity as $CaCO_3$

Table 20 Sample volume selection for expected concentration

Range (mg/L as $CaCO_3$)	Sample Volume (mL)	Sodium Hydroxide	Multiplier
1–1000	50	19353	20
800–2000	25	19353	40
2000–5000	10	19353	100
4000–10000	5	19353	200

Interferences

Highly colored or turbid samples may mask the color change at the end point. Use a pH meter for these samples. Titrate to pH 3.7.

Add a drop of 0.1 N Sodium Thiosulfate Standard Solution to remove residual chlorine that may interfere with the indicator.

Sampling and storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation and prolonged exposure to air. Samples should be analyzed as soon as possible after collection but can be stored at least 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before analyzing.

Accuracy check

End point confirmation

To determine the correct end point, dissolve the contents of a Buffer Powder Pillow, pH 3.7, in 50 mL of deionized water in a 250-mL Erlenmeyer flask. Add the contents of one Bromphenol Blue Indicator Powder Pillow and swirl to mix. Titrate the prepared water test samples to this same color.

Standard additions method (Sample spike)

The standard additions method check can be performed as follows:

4. Use a TenSette Pipet to add 0.1 mL of Sulfuric acid standard solution 0.500 N to a prepared sample titrated to the end point.
5. Swirl to mix and titrate again to the end point. Note the amount of additional titrant used.
6. Make 0.2-mL and 0.3-mL acid additions, titrating to the end point after each addition. The mL of titrant required should increase by 2.5 mL for each 0.1-mL increment of acid added. If these increases do not occur, refer to the Water Analysis Guide for the Standard Additions.

Standard solution method

Sodium Hydroxide Standard Solution slowly absorbs carbon dioxide when exposed to air, causing a partial loss of strength. Check the solution frequently (monthly) by titrating 50 mL of Potassium Acid Phthalate Standard Solution as 100 mg/L CO_2 and using a Bromphenol Blue Indicator Powder Pillow. The titration should require 5.68 mL of Sodium Hydroxide Standard Solution. If the volume required for the titration is greater than 5.88 mL, discard the solution and replace with a fresh supply.

Summary of method

Acidity is classified by the pH value of the titration end point. Various pH indicators are used depending on the pH end point selected. Acidity also can be determined by using a pH meter to follow the solution pH to the correct end point value as the standard base is added.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Catalog number
Bromphenol Blue Powder Pillows	1 pillow	100/pkg	1455099
Sodium Hydroxide Standard Solution, 0.020 N	varies	1 L	19353
Water, Deionized	varies	500 mL	27249

Required apparatus

Description	Quantity/test	Unit	Catalog number
Buret, Teflon Plug, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Select one or more based on sample volume:			
Cylinder, graduated, 5-mL	—	each	50837
Cylinder, graduated, 10-mL	—	each	50838
Cylinder, graduated, 25-mL	—	each	50840
Cylinder, graduated, 50-mL	—	each	50841
Flask, Erlenmeyer, 250-mL	1	each	50546
Pipet, volumetric, Class A, 5 mL	—	each	1451537
Pipet, volumetric, Class A, 10 mL	—	each	1451538
Support Stand	1	each	56300
Funnel, Micro	1	each	2584335

Required standards

Descripiton	Unit	Catalog number
Sulfuric Acid Standard Solution, 0.500 N	100 mL	212132
Buffer Powder Pillows, pH 3.7	25	1455168
Potassium Acid Phthalate Standard Solution, 100-mg/L as CO_2	100 mL	226142

Optional items

Description	Catalog number
Meter, pH, SenION 1 with Platinum pH electrode	5170010
Pipet Filler	1465100
Sodium Thiosulfate Standard Solution, 0.1 N	32332

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Edition 7

Acidity, Phenolphthalein

DOC316.53.01149

Sodium Hydroxide with a Buret[1]

Method 8010

(0 to 10,000 mg/L as $CaCO_3$)

Buret Titration

Scope and Application: For water, wastewater and seawater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater, 23/0 B (4a)*.

Test preparation

Before starting the test:

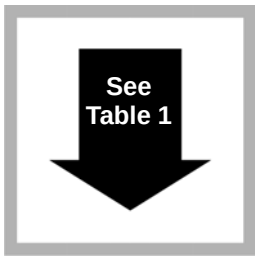
Read the entire procedure before starting the test.
Avoid excessive agitation of the sample to prevent the loss of dissolved gases such as carbon dioxide, hydrogen sulfide or ammonia.
Six drops of Phenolphthalein Indicator Solution may be substituted for the Phenolphthalein Powder Pillow.
The Methyl Orange and Phenolphthalein Acidity procedures can be run sequentially if both values are desired. First, titrate to pH 3.7 and record the amount of sodium hydroxide used. Then titrate to pH 8.3.
Phenolphthalein acidity in mg/L x 0.0584 = grains per gallon

Collect the following items:

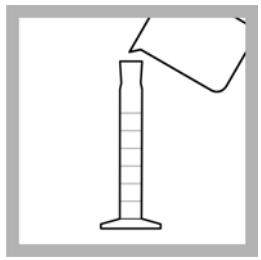
Description	Quantity
Phenolphthalein indicator powder pillow	1
Sodium hydroxide standard solution, 0.020 N	varies[1]
Buret clamp	1
Buret, Teflon plug, Class A	1
Graduated cylinder	varies[1]
Erlenmeyer flask	1
Funnel, Micro	1
Support Stand	1

[1] See *Consumables and replacement items*.

Buret titration (Method 8010)



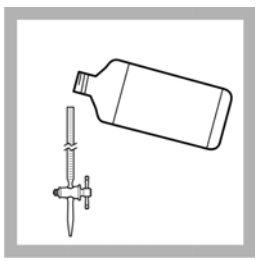
1. Select a sample volume from the *Sample volume selection for expected concentration* table that corresponds to the expected acidity concentration in mg/L as calcium carbonate ($CaCO_3$).



2. Use a graduated cylinder or pipet to measure the sample volume.

3. Transfer the sample into a 250-mL Erlenmeyer flask. Dilute to about 50-mL with deionized water if necessary.

4. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix. (Omit this step if you are using a pH meter.)



5. Fill a 50-mL buret to the zero mark with 0.020 N Sodium Hydroxide Standard Solution.

6. While swirling the flask, titrate the sample with 0.020 N Sodium Hydroxide Standard Solution until the solution color changes from colorless to a light pink that persists for 30 seconds (pH 8.3).

7. Calculate:

mL Titrant × multiplier used = mg/L phenolphthalein acidity as $CaCO_3$

Table 21 Sample volume selection for expected concentration

Range (mg/L as $CaCO_3$)	Sample Volume (mL)	Sodium Hydroxide	Multiplier
1–1000	50	19353	20
800–2000	25	19353	40
2000–5000	10	19353	100
4000–10000	5	19353	200

Interferences

Highly colored or turbid samples may mask the color change at the end point. Use a pH meter for these samples. Titrate to pH 8.3.

Add a drop of 0.1 N Sodium Thiosulfate Standard Solution to remove residual chlorine that may interfere with the indicator.

Pretreat samples that contain significant amounts of hydrolyzable metals such as iron, aluminum or manganese as follows:

1. Adjust the sample taken in step 1 of the procedure to pH 4.0 or less (if necessary) by adding 5.0-mL increments of 0.020 N Sulfuric Acid Standard Solution. Record the amount of acid added. Use a pH meter or appropriate indicator to determine when pH 4.0 is reached. Remove the pH electrodes after completing this step if a pH meter is used.
2. Using a 1-mL glass serological pipet and pipet filler, add five drops of 30% Hydrogen Peroxide Solution.
3. Boil the solution for two to five minutes.
4. Cool to room temperature and titrate to pH 8.3 as described in the procedure beginning with step 3 of the procedure.
5. Subtract the number of milliliters of 0.020 N Sulfuric Acid Standard Solution added from the number of milliliters of 0.020 N Sodium Hydroxide Standard Solution used in the titration before multiplying the volume of sodium hydroxide by the multiplier used in step 7 of the procedure.

Sampling and storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation and prolonged exposure to air. Samples should be analyzed as soon as possible after collection but can be stored at least 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before analyzing.

Accuracy check

End point confirmation

To determine the correct end point, dissolve the contents of a Buffer Powder Pillow, pH 8.3, in 50 mL of deionized water in a 250-mL Erlenmeyer flask. Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix. Titrate the prepared water test samples to this same color.

Standard additions method (Sample spike)

The standard additions method check can be performed as follows:

1. Use a TenSette Pipet to add 0.1 mL of Sulfate standard solution 0.500 N to a prepared sample titrated to the end point.
2. Swirl to mix and titrate again to the end point. Note the amount of additional titrant used.
3. Make 0.2-mL and 0.3-mL acid additions, titrating to the end point after each addition. The mL of titrant required should increase by 2.5 mL for each 0.1-mL increment of acid added. If these increases do not occur, refer to the Water Analysis Guide for Standard Additions.

Standard solution method

Sodium Hydroxide Standard Solution slowly absorbs carbon dioxide when exposed to air, causing a partial loss of strength. Check the solution frequently (monthly) by titrating 50 mL of Potassium Acid Phthalate Standard Solution as 100 mg/L $CO_2$and using a Phenolphthalein Indicator Powder Pillow. The titration should require 5.68 mL of Sodium Hydroxide Standard Solution. If the volume required for the titration is greater than 5.88 mL, discard the solution and replace with a fresh supply.

Summary of method

Acidity is classified by the pH value of the titration end point. Various pH indicators are used depending on the pH end point selected. Acidity also can be determined by using a pH meter to follow the solution pH to the correct end point value as the standard base is added.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Catalog number
Phenolphthalein Powder Pillows	1 pillow	100/pkg	94299
Sodium Hydroxide Standard Solution, 0.020 N	varies	1 L	19353
Water, Deionized	varies	500 mL	27249

Required apparatus

Description	Quantity/test	Unit	Catalog number
Buret, Teflon Plug, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Select one or more based on sample volume:			
Cylinder, graduated, 5-mL	—	each	50837
Cylinder, graduated, 10-mL	—	each	50838
Cylinder, graduated, 25-mL	—	each	50840
Cylinder, graduated, 50-mL	—	each	50841
Flask, Erlenmeyer, 250-mL	1	each	50546
Pipet, volumetric, Class A, 5 mL	—	each,	1451537
Pipet, volumetric, Class A, 10 mL	—	each	1451538
Pipet Filler, Safety bulb	—	each	1465100
Support Stand	1	each	56300
Funnel, Micro	1	each	2584335

Required standards

Description	Unit	Catalog number
Sulfuric Acid Standard Solution, 0.500 N	100 mL MDB	212132
Buffer Powder Pillows, pH 8.3, 50 mL	25/pkg	89868
Potassium Acid Phthalate Standard Solution, 100-mg/L as CO_2	100 mL	226142

Optional items

Description	Unit	Catalog number
Hydrogen Peroxide Solution. 30 %	473 mL	14411
Meter, pH, SensION 1 with Platinum pH electrode	each	5170010
Phenolphthalein Indicator Solution	100 mL MDB	16232
Pipet, 1-mL glass serological pipet	each	53235
Pipet Filler	each	1465100
Sodium Thiosulfate Standard Solution, 0.1 N	100 mL MDB	32332

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Edition 7

Acidity

DOC316.53.01164

Methyl Orange and Phenolphthalein (Total) Acidity

Method 8201 and 8202

10 to 4000 mg/L $CaCO_3$

Digital Titrator

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

Avoid excess agitation when collecting and swirling the sample to prevent the loss of gases such as carbon dioxide, hydrogen sulfide and ammonia.
Six drops of Bromphenol Blue Indicator Solution[1] can be substituted for the Bromphenol Blue Indicator Powder Pillow.
Four drops of Phenolphthalein Indicator Solution[1] can be substituted for the Phenolphthalein Indicator Powder Pillow.
A pH meter can be used in place of the indicators. The end point for methyl orange acidity is pH 3.7. The end point for phenolphthalein acidity is pH 8.3.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

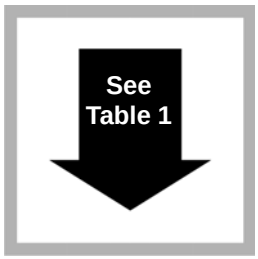
[1] See *Optional reagents and apparatus*.

Collect the following items:

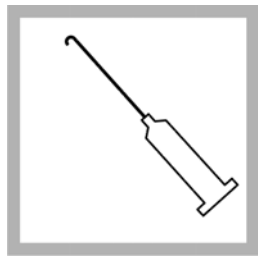
Description	Quantity
Bromphenol Blue Indicator Powder Pillow	1 pillow
Phenolphthalein Indicator Powder Pillow	1 pillow
Sodium Hydroxide Titration Cartridge (see *Range-specific information*)	1 cartridge
Digital Titrator	1
Delivery Tube for Digital Titrator	1
Graduated Cylinder	1
Erlenmeyer Flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Methyl orange acidity (Method 8201)

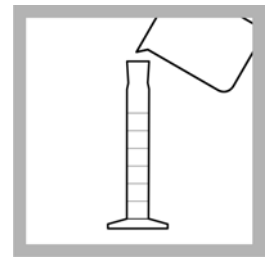


1. Select a sample volume and titration cartridge from the *Range-specific information*.



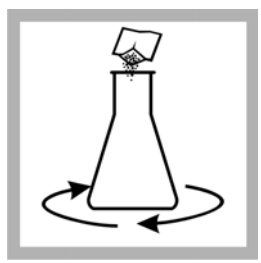
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.



4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information*.

5. Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.



6. Add the contents of one Bromphenol Blue Indicator Powder Pillow. Swirl to mix.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from yellow to blue-violet (pH 3.7).

Write down the number of digits displayed on the counter.

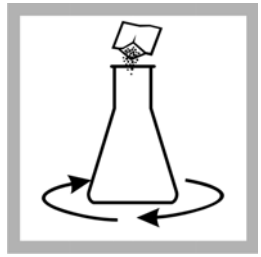
8. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L as $CaCO_3$ methyl orange acidity

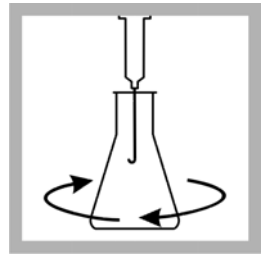
Example: 100 mL of sample was titrated with the 0.1600 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 mg/L as $CaCO_3$

Phenolphthalein (total) acidity (Method 8202)

1. Measure a second portion of the sample from the *Range-specific information* table into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.



2. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix.



3. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from colorless to a light pink color that persists for 30 seconds.

Write down the number of digits displayed on the counter.

4. Use the multiplier in *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L as $CaCO_3$ phenolphthalein acidity

Example: 100 mL of sample was titrated with the 1.600 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 1.0 = 250 mg/L as $CaCO_3$

Table 22 Range-specific information

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Titration cartridge (N NaOH)	Multiplier
10–40	100	0.1600	0.1
40–160	25	0.1600	0.4
100–400	100	1.600	1.0
200–800	50	1.600	2.0
500–2000	20	1.600	5.0
1000–4000	10	1.600	10.0

Interferences

Interfering substances lists substances that can interfere with this test.

Table 23 Interfering substances

Interfering substance	Interference level
Chlorine	Chlorine may interfere with the indicators. Add one drop of 0.1 N Sodium Thiosulfate to the sample to remove chlorine before starting the test.
Color or turbidity	Color or turbidity can mask the color change of the end point. Use a pH meter instead of the color indicators and titrate to a pH of 3.7 for methyl orange acidity or pH 8.3 for phenolphthalein acidity.

Table 23 Interfering substances (continued)

Interfering substance	Interference level
Hydrolyzable metals	Samples that contain hydrolyzable metals such as iron, manganese or aluminum should be pretreated before the test for phenolphthalein acidity is started. **1.** Measure the sample volume into the flask. **2.** Use the Digital Titrator and a Sulfuric acid cartridge of the same normality as the one being used for the Acidity test to adjust the sample pH to pH 4 or less. Write down the number of digits of acid that was added to lower the pH. **3.** Add five drops of 30% hydrogen peroxide solution. **4.** Boil the solution for 2–5 minutes. **5.** Cool the solution to room temperature. **6.** Add the phenolphthalein indicator and titrate the sample. **7.** Subtract the number of digits of acid that were added in step 2 from the number of digits that were used to reach the end point in step 6. Multiply this number by the multiplier in *Range-specific information* to find the phenolphthalein acidity in the sample.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Fill completely and cap tightly.
- Prevent excessive agitation or prolonged exposure to air.
- Complete the test procedure as soon as possible after collection for best accuracy. The sample can be stored for at least 24 hours if cooled to 4 °C (39 °F) or below.
- Warm to room temperature before the test is started.

Accuracy check

End point confirmation
Use a buffer pillow with the same pH as the end point with the indicator to make sure the end point color is accurate.

- Methyl orange acidity—Add 50 mL of deionized water to a flask. Add one pH 3.7 buffer powder pillow and one Bromphenol Blue Indicator Powder Pillow and swirl to mix. Use this solution for comparison during the titration with the sample.
- Phenolphthalein acidity—Add 50 mL of deionized water to a flask. Add one pH 8.3 buffer powder pillow and one Phenolphthalein Indicator Powder Pillow and swirl to mix. Use this solution for comparison during the titration with the sample.

Standard additions method (sample spike)
Required for accuracy check:

- Sulfuric Acid Standard Solution, 0.500 N
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL

1. Open the standard solution.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 0.1 mL of standard that was added will use approximately 25 digits of the 1.600 N titration cartridge or 250 digits of the 0.1600 N titration cartridge to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed.

Summary of method

Bromphenol blue or phenolphthalein indicator is used to titrate the sample with sodium hydroxide to a colorimetric end point. Bromphenol blue gives a better end point than methyl orange indicator. Titration to pH 3.7 determines strong mineral acidity (also referred to as methyl orange acidity), whereas the pH 8.3 phenolphthalein end point includes weaker acid species as well and represents the total acidity. The results are expressed in mg/L as calcium carbonate ($CaCO_3$) at a specified pH.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Acidity Reagent Set (approximately 100 tests)			2272800
(1) Bromphenol Blue Powder Pillows	1 pillow	100/pkg	1455099
(1) Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
(1) Sodium Hydroxide Titration Cartridge, 0.1600 N	varies	each	1437701
(1) Sodium Hydroxide Titration Cartridge, 1.600 N	varies	each	1437901

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842

Recommended standards

Description	Unit	Catalog number
SUlfuric Acid Standard Solution, 0.500 N H_2SO_4, 10-mL	100 mL	212132

Optional reagents and apparatus

Description	Unit	Catalog number
Buffer Powder Pillows, pH 3.7	25/pkg	1455168
Buffer Powder Pillows, pH 8.3	25/pkg	89868
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Pipet tips	50/pkg	2185696
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Sulfuric Acid DT cartridge, 0.1600	each	1438801
Sulfuric Acid DT cartridge, 1.600	each	1438901
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet, volumetric, Class A, 20 mL	each	1451520
Pipet Filler, safety bulb	each	1465100
Bottles, sampling, poly, 500 mL	each	2087079
Bromphenol Blue indicator solution	100 mL MDB	1455232
Phenolphthalein Indicator solution, 5 g/L	100 mL MDB	16232
pH meter	each	—
Delivery tube, 180° hook	5/pkg	1720500

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Edition 7

Scope and Application: For water

[1] This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 24 Instrument-specific information

Instrument	Adapter
DR 6000	—
DR 5000	A23618
DR 3900	LZV846(A)
DR3800, DR 2800, DR 2700	LZV583

Before starting the test:

This method analyzes for Alachlor in water. Sample calibrators and reagents are added to cuvettes coated with Alachlor-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Read the entire procedure before starting. Identify and make ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.

Timing is critical; follow instructions carefully.

A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.

Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.

Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.

To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.

The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the spectrophotometer.

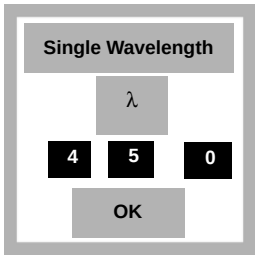
Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.

Protective nitrile gloves are recommended for this procedure.

Collect the following items:

Description	Quantity
Alachlor Reagent Set	1
Caps, flip spout	1
Marker, laboratory	1
Rack, for 1-cm Micro Cuvettes	1
Wipes, disposable	1
Pipet, TenSette®, 0.1–1.0 mL	1
Pipet tip for 19700-01, TenSette Pipet	1

Immunoassay for Water

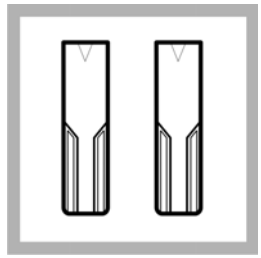


1. Press **SINGLE WAVELENGTH**

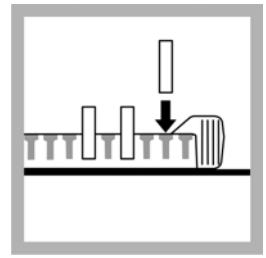
Press **OPTIONS** and the λ button.

Enter **450 NM** and press **OK**.

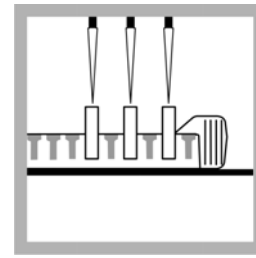
Insert an adapter if required (*Instrument-specific information*). Refer to the user manual for orientation.



2. Label an Antibody Cuvette for each calibrator and each sample to be tested.



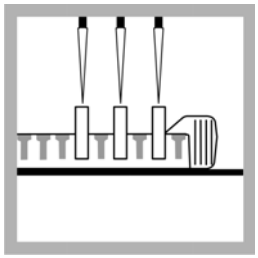
3. Insert the cuvettes into the rack snugly.



4. Pipet 0.5 mL of each **calibrator** into the appropriately labeled cuvette.

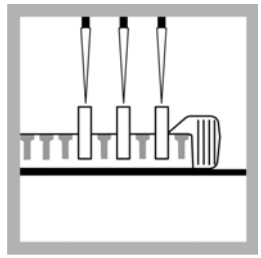
Use a new pipette tip for each calibrator.

Immunoassay for Water (continued)



5. Pipet 0.5 mL of each **sample** to be tested into the appropriately labeled cuvette.

Use a new pipette tip for each sample.

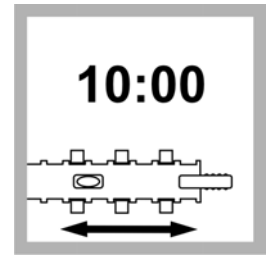


6. Immediately pipet 0.5 mL of Alachlor Enzyme Conjugate into each cuvette.

Use the same pipette tip for each sample.

7. Set the instrument timer for 20:00 minutes. Press **OK** to start a 20-minute reaction time.

Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



8. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

9. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

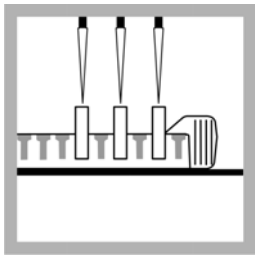
10. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Ensure that most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.

Immunoassay for Water (continued)

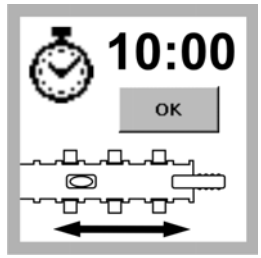
Color Development

Important Note: *Timing is critical. Follow instructions carefully.*



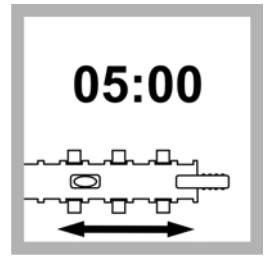
11. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Use the same pipette tip for each sample.



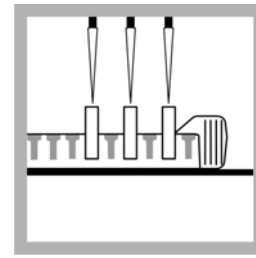
12. Set the instrument timer for 10:00 minutes. Press **OK** to start the reaction period.

Mix, using the instructions in *Using the 1-cm MicroCuvette Rack*.



13. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique as step 12.

Solutions will turn blue in some or all of the cuvettes.



14. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 11.

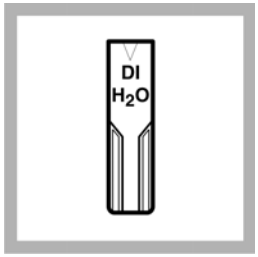
Slide the rack for 20 seconds (*Using the 1-cm MicroCuvette Rack*.)

Blue solutions will turn yellow with the addition of the Stop Solution.

Use the same pipette tip repeatedly for this step.

Immunoassay for Water (continued)

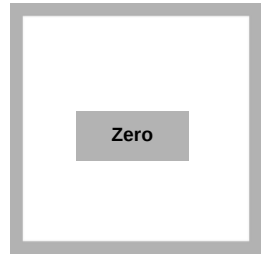
Measuring the Color



15. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

16. Insert the filled Zeroing Cuvette into the cell holder.

Orient the arrow in the same direction for all cuvettes.



17. **ZERO** the instrument.

The display will show: 0.000 Abs

18. Insert the first calibrator into the cell holder.

READ the results in (ABS) for each calibrator and sample. Record the results.

Repeat this step for all remaining calibrators and samples.

See *Interpreting and reporting results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

The MicroCuvette rack (Figure 1) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

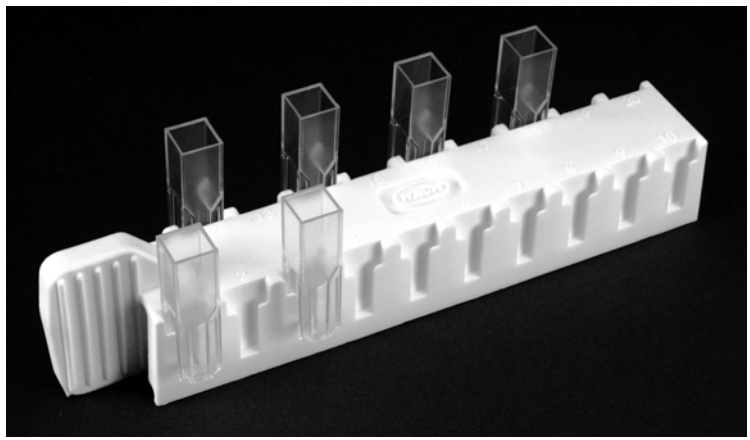


Figure 1 The 1-cm MicroCuvette Rack

Loading the rack—The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and insert all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing—Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and reporting results

There is an inverse relationship between the concentration of Alachlor and the absorbance reading. In other words, the higher the reading, the lower the concentration of Alachlor (see *Relative Alachlor concentration*)

Table 25 Relative Alachlor concentration

If the sample reading is...	the sample Alachlor Concentration is...
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example
Readings:

0.1 ppb Alachlor Calibrator: **0.475 Abs**

0.5 ppb Alachlor Calibrator: **0.245 Abs**

Sample #1: **0.140 Abs**

Sample #2: **0.300 Abs**

Sample #3: **0.550 Abs**

Interpretation
Sample #1—Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Alachlor is greater than both 0.1 ppb and 0.5 ppb Alachlor.

Sample #2—Sample reading is between the readings for the 0.1 ppb and 0.5 ppb Alachlor calibrators. Therefore the sample concentration of Alachlor is between 0.1 ppb and 0.5 ppb.

Sample #3—Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Alachlor is less than both 0.5 ppb and 0.1 ppb.

Storing and handling reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Alachlor immunoassay test cannot differentiate between certain herbicides and metabolites, but it detects their presence to differing degrees. The *Required concentration for selected chemicals* table shows the required concentration for selected chemicals.

Table 26 Required concentration for selected chemicals

Compound	Concentration to give a positive response of 0.1 ppb Alachlor	Concentration to give a positive response of 0.5 ppb Alachlor
Acetochlor	0.45 ppb	4 ppb
Butachlor	0.09 ppm	1 ppm
2 Chloro-2',6'-Diethylacetaniline	0.030 ppm	2 ppm
Metolachlor	0.085 ppm	2 ppm
2,6-Diethylaniline	0.3 ppm	9 ppm
Propachlor	0.72 ppb	12 ppb

Table 27 Compounds tested and not detected up to 10,000 ppb

Atrazine	2, 4-D
Aldicarb	Chlorpyrifos
Diazoton	Carbendazim
Carbofuran	

Sample collection and storage

Collect samples in a clean glass bottle. **Do not pre-rinse the bottle with the sample.** If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Water sample dilution

Other levels of Alachlor can be tested by diluting the sample and comparing the results to the 0.1 ppb Calibrator. Select the appropriate sample volume from the *Sample volume and concentration* table, place it in a graduated mixing cylinder, and dilute it to 50 mL with deionized water.

Table 28 Sample volume and concentration

mL sample	Representative concentration using 0.1 ppb calibrator
0.5	10 ppb
1.0	5 ppb
2.5	2 ppb
5.0	1 ppb

Example:
Dilute 2.5 mL of sample to 50 mL with deionized water. Run the diluted sample in the procedure along with the 0.1 ppb calibrator. If the absorbance of the diluted sample is less than the 0.1 ppb calibrator, the concentration of the original sample is greater than 2 ppb.

Summary of method

Immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Alachlor-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Alachlor from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Alachlor compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Alachlor and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Alachlor in the sample. The resulting color is then compared with a calibrator to determine whether the Alachlor concentration in the sample is greater or less than the threshold levels. Test results are measured at 450 nm.

Consumables and replacement items

Required reagents

Description	Unit	Catalog Number
Alachlor Reagent Set[1]	20 cuvettes	2813000

[1] Immunoassay components are manufactured by Beacon Analytical Systems, Inc.

Required apparatus

Description	Unit	Catalog Number
Caps, flip spout	2/pkg	2581802
Marker, laboratory	each	2092000
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Rack, for 1-cm Micro Cuvettes	each	4879900
Wipes, disposable	box	2097000

Optional reagents and apparatus

Description	Unit	Catalog Number
Deionized Water[1]	500 mL	27249
Glasses, Safety	each	2756800
Gloves, Disposable, Nitrile, Medium[1]	each	2550502
Mixing Cylinder, graduated Class A, 50-mL[1]	each	2636341
Mixing Cylinder, graduated Class A, 25-mL[1]	each	2636340
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	2185696

[1] Other sizes available.

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Edition 7

Alkalinity

DOC316.53.01166

Phenolphthalein and Total Alkalinity

Method 8203

10 to 4000 mg/L as $CaCO_3$

Digital Titrator

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

Four drops of Bromcresol Green-Methyl Red Indicator Solution[1] can be substituted for the Bromcresol Green-Methyl Red Indicator Powder Pillow.
Four drops of Phenolphthalein Indicator Solution[1] can be substituted for the Phenolphthalein Indicator Powder Pillow.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].
meq/L Alkalinity = mg/L as $CaCO_3 \div 50$

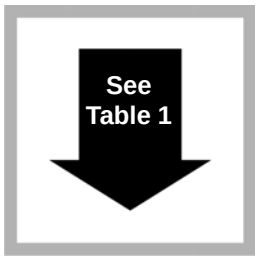
[1] See *Optional reagents and apparatus*.

Collect the following items:

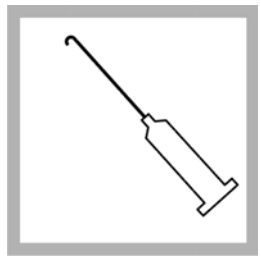
Description	Quantity
Bromcresol Green-Methyl Red Indicator Powder Pillow	1 pillow
Phenolphthalein Indicator Powder Pillow	1 pillow
Sulfuric acid titration cartridge (see *Range-specific information*)	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Alkalinity

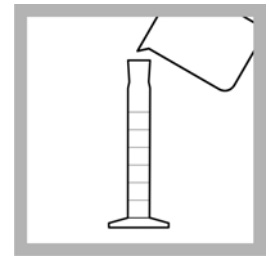


1. Select a sample volume and titration cartridge from the *Range-specific information* table.



2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

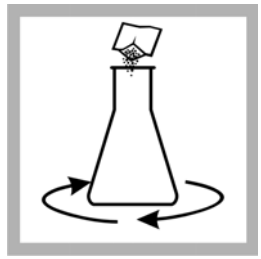
3. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.



4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table.

Alkalinity (continued)

5. Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.



6. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix.

If the solution turns pink, proceed to step 7. If the solution is colorless, the Phenolphthalein (P) alkalinity is zero. Proceed to step 9.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from pink to colorless.

Write down the number of digits displayed on the counter.

8. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L as $CaCO_3$
P alkalinity

Example: 100 mL of sample was titrated with the 0.1600 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 mg/L as $CaCO_3$

9. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow. Swirl to mix.

10. Continue the titration with sulfuric acid to a light pink color.

Write down the number of digits displayed on the counter.

Note: A pH meter may be used to titrate to a specific pH as required by sample composition. See the *End point pH* table.

11. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L as $CaCO_3$
total alkalinity

Example: 100 mL of sample was titrated with the 0.1600 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 mg/L as $CaCO_3$

Table 29 Range-specific information

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Titration cartridge (N H_2SO_4)	Multiplier
10–40	100	0.1600	0.1
40–160	25	0.1600	0.4
100–400	100	1.600	1.0
200–800	50	1.600	2.0
500–2000	20	1.600	5.0
1000–4000	10	1.600	10.0

Table 30 End point pH

Sample composition	Total alkalinity	Phenolphthalein alkalinity
Alkalinity about 30 mg/L	pH 4.9	pH 8.3
Alkalinity about 150 mg/L	pH 4.6	pH 8.3
Alkalinity about 500 mg/L	pH 4.3	pH 8.3
Silicates or phosphates present	pH 4.5	pH 8.3
Industrial wastes or complex system	pH 4.5	pH 8.3
Routine or Automated Analyses	pH 4.5	pH 8.3

Interferences

Interfering substances lists substances that can interfere with this test.

Table 31 Interfering substances

Interfering substance	Interference level
Chlorine	Chlorine at levels above 3.5 mg/L may cause a yellow-brown color when the Bromcresol Green-Methyl Red Powder Pillow is added. Add one drop of 0.1 N Sodium Thiosulfate to the sample to remove chlorine before starting the test.
Color or turbidity	Color or turbidity can mask the color change of the end point. Use a pH meter instead of the color indicators and titrate to a pH of 8.3 for phenolphthalein acidity. For total alkalinity see *End point pH* for the correct end point pH.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Fill completely and cap tightly.
- Prevent excessive agitation or prolonged exposure to air. Complete the test procedure as soon as possible after collection for best accuracy.
- The sample can be stored for at least 24 hours if cooled to 4 °C (39 °F) or below.
- Warm to room temperature before the test is started.

Alkalinity relationship table

Total alkalinity primarily includes hydroxide, carbonate and bicarbonate alkalinities. The concentration of these alkalinities in a sample may be determined when the phenolphthalein and total alkalinities are known (see *Alkalinity relationships*).

To use the table follow these steps:

g. Does the phenolphthalein alkalinity equal zero? If yes, use Row 1.

h. Does the phenolphthalein alkalinity equal total alkalinity? If yes, use Row 2.

i. Divide the total alkalinity by 2 to give one-half the total alkalinity.

j. Select Row 3, 4 or 5 based on comparing the result of step c (one-half total alkalinity) with the total alkalinity.

k. Perform the required calculations in the appropriate row, if any.

l. Check your results. The sum of the three alkalinity types will equal the phenolphthalein alkalinity.

For example:
A sample has 170 mg/L as $CaCO_3$ phenolphthalein alkalinity and 250 mg/L as $CaCO_3$ total alkalinity. What is the concentration of hydroxide, carbonate and bicarbonate alkalinities?

The phenolphthalein alkalinity does not equal 0 (it is 170 mg/L), see step g.

The phenolphthalein alkalinity does not equal total alkalinity (170 mg/L vs. 250 mg/L), see step h.

One-half of the total alkalinity (250 g/L) equals 125 mg/L. Because the phenolphthalein alkalinity (170 mg/L) is greater than one-half the total alkalinity (125 mg/L), select row 5.

The hydroxide alkalinity is equal to:
2 x 170 = 340

340 – 250 = 90 mg/L hydroxide alkalinity

The carbonate alkalinity is equal to:
250 – 170 = 80

80 x 2 = 160 mg/L carbonate alkalinity

The bicarbonate alkalinity equals 0 mg/L.
Check: (See step l)

90 mg/L hydroxide alkalinity + 160 mg/L carbonate alkalinity + 0 mg/L bicarbonate alkalinity = 250 mg/L

The above answer is correct; the sum of each type equals the total alkalinity.

Table 32 Alkalinity relationships

Row	Sample result	Hydroxide alkalinity equals:	Carbonate alkalinity equals:	Bicarbonate alkalinity equals:
1	Phenolphthalein Alkalinity = 0	0	0	Total Alkalinity
2	Phenolphthalein Alkalinity equal to Total Alkalinity	Total Alkalinity	0	0
3	Phenolphthalein Alkalinity less than one-half of Total Alkalinity	0	Phenolphthalein Alkalinity times 2	Total Alkalinity minus two times Phenolphthalein Alkalinity
4	Phenolphthalein Alkalinity equal to one-half of Total Alkalinity	0	Total Alkalinity	0
5	Phenolphthalein Alkalinity greater than one-half of Total Alkalinity	2 times Phenolphthalein Alkalinity minus Total Alkalinity	2 times the difference between Total and Phenolphthalein Alkalinity	0

Accuracy check

End point confirmation
Use a buffer pillow with the same pH as the end point with the indicator to make sure the end point color is accurate.

- Phenolphthalein alkalinity—Add 50 mL of deionized water to a flask. Add one pH 8.3 buffer powder pillow and one Phenolphthalein Indicator Powder Pillow and swirl to mix. Use this solution for comparison during the titration with the sample.
- Total alkalinity—Add 50 mL of deionized water to a flask. Add one pH 4.5 buffer powder pillow and one Bromcresol Green-Methyl Red Indicator Powder Pillow and swirl to mix. Use this solution for comparison during the titration with the sample.

Standard additions method (sample spike)
Required for accuracy check:

- Alkalinity Voluette® Ampule Standard Solution, 0.500 N
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL and Pipet Tips

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Repeat steps 2 and 3, using two more additions of 0.1 mL. Titrate to the end point after each addition.
5. Each 0.1 mL of standard that was added will use approximately 25 digits of the 1.600 N titration cartridge or 250 digits of the 0.1600 N titration cartridge to reach the endpoint. If more or less titrant was used, there may be an interference (see *Interferences*) or the concentration of the titrant has changed.

Summary of method

The sample is titrated with sulfuric acid to a colorimetric end point corresponding to a specific pH. Phenolphthalein alkalinity is determined by titration to a pH of 8.3, as evidenced by the color change of phenolphthalein indicator and indicates the total hydroxide and one half the carbonate present. M (methyl orange) or T (total) alkalinity is determined by titration to a pH between 4.3 and 4.9 and includes all carbonate, bicarbonate and hydroxide. Alternatively, total alkalinity end points may be determined by using a pH meter and titrating to the specific pH required for the sample composition.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Alkalinity Reagent Set (approximately 100 tests)			2271900
(1) Bromcresol Green-Methyl Red Powder Pillows	1 pillow	100/pkg	94399
(1) Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
(1) Sulfuric Acid Titration Cartridge, 0.1600 N	varies	each	1438801
(1) Sulfuric Acid Titration Cartridge, 1.600 N	varies	each	1438901

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator		each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842

Recommended standards

Description	Unit	Catalog number
Alkalinity Standard Solution, Voluette® Ampule 0.500 N Na_2CO_3, 10-mL	16/pkg	1427810

Optional reagents and apparatus

Description	Unit	Catalog number
Buffer Powder Pillows, pH 4.5	25/pkg	89568
Buffer Powder Pillows, pH 8.3	25/pkg	89868
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Water, deionized	500 mL	27249
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet, volumetric, Class A, 20 mL	each	1451520
Pipet Filler, safety bulb	each	1465100
Bottles, sampling, poly, 500 mL	each	2087079
Bromphenol Green-Methyl Red indicator solution	100 mL MDB	2329232
Phenolphthalein Indicator solution, 5 g/L	100 mL MDB	16232
pH meter	each	—
TitraStir stir plate, 115 Vac	each	1940000
TitraStir stir plate, 230 Vac	each	1940010

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Edition 7

Alkalinity

DOC316.53.01151

USEPA[1] Buret Titration Method[2]

Method 8221

(0 to 5,000 mg/L as $CaCO_3$)

Buret Titration

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater, 2320 B*

Test preparation

Before starting the test:

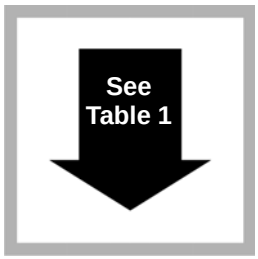
Read the entire procedure before starting the test.
A pH meter is required for NPDES reporting and is recommended for best results.
Substitute six drops of Phenolphthalein Indicator Solution for the Phenolphthalein Indicator Powder Pillow if necessary
Substitute six drops of Bromcresol Green-Methyl Red Indicator Solution for the Bromcresol Green-Methyl Red Powder Pillow if necessary.
Results in mg/L as $CaCO_3$ ÷ 17.12 = grains per gallon

Collect the following items:

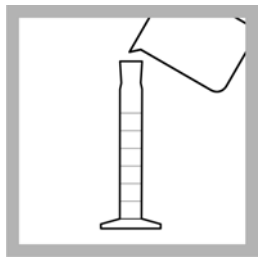
Description	Quantity
Bromcresol Green-Methyl Red indicator powder pillow	1
Phenolphthalein indicator powder pillow	1
Sulfuric acid standard solution, 0.020 N	varies[1]
Buret clamp, double	1
Buret, Class A, 25-mL	1
Graduated cylinder	varies[1]
Erlenmeyer flask, 250-mL	1
Funnel, Micro	1
Support Stand	1

[1] See *Consumables and replacement items* on page 116.

Buret titration (Method 8221)



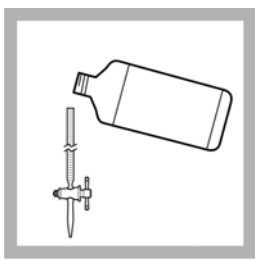
1. Select a sample volume from the *Sample volume selection for expected concentration* table that corresponds to the expected alkalinity concentration in mg/L as calcium carbonate ($CaCO_3$).



2. Use a graduated cylinder or pipet to measure the sample volume.

3. Transfer the sample into a 250-mL Erlenmeyer flask. Dilute to about 50-mL with deionized water if necessary.

4. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix. (Omit this step when using a pH meter.)



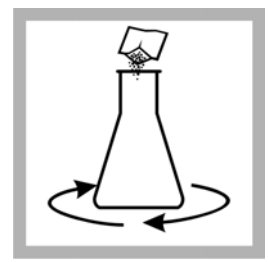
5. Fill a 25-mL buret to the zero mark with 0.020 N Sulfuric Acid standard solution.

6. While swirling the flask, titrate the sample until the solution color changes from pink to colorless (pH 8.3).

If the solution is colorless before titrating with sulfuric acid, the phenolphthalein alkalinity is zero.

7. Calculate:

mL Titrant × multiplier used = mg/L phenolphthalein alkalinity as $CaCO_3$.



8. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the titrated sample. Swirl to mix.

Do not add indicator if a pH meter is used.

Specific sample composition may require titration to a specific pH (see the *Alkalinity relationship* table).

Buret titration (Method 8221)

9. Continue the titration until a light pink end point is reached.

10. Calculate:

mL Titrant × multiplier used = mg/L total alkalinity as $CaCO_3$.

Table 33 Sample volume selection for expected concentration

Range (mg/L as $CaCO_3$)	Sample Volume (mL)	Sulfuric Acid	Multiplier
0–500	50	20353	20
400–1000	25	20353	40
1000–2500	10	20353	100
2000–5000	5	20353	200

The end points in the *Alkalinity endpoints* table are recommended for determining total alkalinity in water samples of various compositions and alkalinity concentrations.

Table 34 Alkalinity endpoints

Sample composition	End point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity about 30 mg/L	pH 4.9	pH 8.3
Alkalinity about 150 mg/L	pH 4.6	pH 8.3
Alkalinity about 500 mg/L	pH 4.3	pH 8.3
Silicates or phosphates present	pH 4.5	pH 8.3
Industrial wastes or complex system	pH 4.5	pH 8.3
Routine or Automated Analyses	pH 4.5	pH 8.3

Total alkalinity primarily includes hydroxide, carbonate, and bicarbonate alkalinities. The concentration of these types in a sample may be determined when the phenolphthalein and total alkalinities are known (*Alkalinity relationship* table).

Table 35 Alkalinity relationship

Row	Result of Titration	Hydroxide Alkalinity Equals:	Carbonate Alkalinity Equals:	Bicarbonate Alkalinity Equals:
1	Phenolphthalein Alkalinity equal to 0	0	0	Total Alkalinity
2	Phenolphthalein Alkalinity equal to Total Alkalinity	Total Alkalinity	0	0
3	Phenolphthalein Alkalinity less than one-half of Total Alkalinity	0	Phenolphthalein Alkalinity times 2	Total Alkalinity minus two times Phenolphthalein Alkalinity
4	Phenolphthalein Alkalinity equal to one-half of Total Alkalinity	0	Total Alkalinity	0
5	Phenolphthalein Alkalinity greater than one-half of Total Alkalinity	2 times Phenolphthalein Alkalinity minus Total Alkalinity	2 times the difference between Total and Phenolphthalein Alkalinity	0

Use the *Alkalinity relationship* table with the following procedure:

1. Does the phenolphthalein alkalinity equal zero? If yes, use Row 1.
2. Does the phenolphthalein alkalinity equal total alkalinity? If yes, use Row 2.
3. Divide the total alkalinity by 2 to calculate one-half the total alkalinity.
4. Select Row 3, 4 or 5 based on comparing the result of step c (one-half total alkalinity) with the phenolphthalein alkalinity.
5. Perform the required calculations if any.
6. Check your results. The sum of the three alkalinity types will equal the total alkalinity.

Example:
A sample has 170 mg/L as $CaCO_3$ phenolphthalein alkalinity and 250 mg/L as CaCO3 total alkalinity. What is the concentration of hydroxide, carbonate, and bicarbonate alkalinities?

- **a.** The phenolphthalein alkalinity does not equal zero but 170 mg/L.
- **b.** The phenolphthalein alkalinity does not equal total alkalinity (170 mg/L vs. 250 mg/L).
- **c.** One-half of the total alkalinity equals 125 mg/L.
- **d.** Because the phenolphthalein alkalinity of 170 mg/L is greater than one-half the total alkalinity of 125 mg/L, select Row 5.

The hydroxide alkalinity is equal to:

$$2 \times 170 = 340$$

$$340 - 250 = 90 \text{ mg/L hydroxide alkalinity}$$

The carbonate alkalinity is equal to:

$$250 - 170 = 80$$

$$80 \times 2 = 160 \text{ mg/L carbonate alkalinity}$$

The bicarbonate alkalinity is equal to zero mg/L.

Check:

90 mg/L hydroxide alkalinity + 160 mg/L carbonate alkalinity + 0 mg/L bicarbonate alkalinity = 250 mg/L

The answer is correct.

The sum of each type equals the total alkalinity (250 mg/L).

Interferences

Chlorine at levels above 3.5 mg/L cause a yellow-brown color upon the addition of the Bromcresol Green-Methyl Red Indicator Powder Pillow. Residual chlorine interference with the indicator may be removed by adding a drop of 0.1 N Sodium Thiosulfate Standard Solution* before adding the indicator.

Highly colored or turbid samples may mask the color change at the end point. Use a pH meter for these samples, titrating to pH 8.3 for phenolphthalein alkalinity and the appropriate pH (see the *Alkalinity endpoints* table) for total alkalinity.

Sampling and storage

Collect samples in plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation and prolonged exposure to air. Samples should be analyzed as soon as possible after collection but can be stored at least 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before analyzing.

Accuracy check

End point confirmation

- To accurately determine the phenolphthalein alkalinity end point, mix the contents of one Phenolphthalein Indicator Powder Pillow and the contents of one pH 8.3 Buffer Powder Pillow with 50 mL of deionized water in a 250-mL Erlenmeyer flask. The resulting color is the end point.
- To accurately determine the total alkalinity end point, mix the contents of one pH 4.5 Buffer Powder Pillow and the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow with 50 mL of deionized water in a 250-mL Erlenmeyer flask. Titrate to a light pink color change.

Standard additions method (Sample Spike)
Perform the standard additions method check as follows:

1. Break the top off an Alkalinity Voluette® Ampule Standard Solution, 0.500 N.
2. Use the TenSette Pipet* to add 0.1 mL of standard to the sample titrated in step 6 or step 9. Resume titration back to the same end point. Record the volume of titrant needed.
3. Repeat, using two more additions of 0.1 mL. Titrate to the end point after each addition.
4. The mL of titrant required should increase by 2.5 mL for each 0.1 mL increment of standard added.

Summary of method

Alkalinity is expressed as P (phenolphthalein) alkalinity or as T (total) alkalinity. Both types are determined by titration with a Sulfuric Acid Standard Solution to an end point evidenced by the color change of an indicator solution or determined with a pH meter. The P alkalinity is determined by titration to a pH of 8.3 and registers the total hydroxide and one half the carbonate present. The T alkalinity is determined by titration to a pH of 4.5. The total alkalinity includes all carbonate, bicarbonate and hydroxide alkalinity. Alternatively, total alkalinity end points may be determined by using a pH meter and titrating to the specific pH required for the sample composition.

* See *Consumables and replacement items* on page 116.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Catalog number
Bromcresol Green-Methyl Red Indicator Powder Pillows	1 pillow	100/pkg	94399
Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
Sulfuric Acid Standard Solution, 0.020 N	varies	1 L	20353

Required apparatus

Description	Quantity/test	Unit	Catalog number
Buret Clamp, double	1	each	32800
Buret, Class A, 25-mL	1	each	2636540
Select one or more based on sample volume:			
Cylinder, graduated, 5-mL	—	each	50837
Cylinder, graduated, 10-mL	—	each	50838
Cylinder, graduated, 25-mL	—	each	50840
Cylinder, graduated, 50-mL	—	each	50841
Flask, Erlenmeyer, 250-mL	1	each	50546
Pipet, volumetric, Class A, 5-mL,	—	each	1451537
Pipet, volumetric, Class A, 10-mL	—	each	1451537
Pipet Filler, Safety Bulb	—	each	1465100
Ampule Breaker	—	each	2196800
Funnel, Micro	1	each	2584335
Support Stand	1	each	56300

Required standards

Description	Unit	Catalog number
Alkalinity Standard Solution, Voluette® Ampules, 0.500 N, 10-mL	16/pkg	1427810
Buffer Powder Pillows, pH 4.5	25/pkg	89568
Buffer Powder Pillows, pH 8.3	25/pkg	89868
Water, deionized	4 L	27256

Optional items

Description	Unit	Catalog number
Sodium Thiosulfate Standard Solution, 0.1 N	—	32332
TenSette Pipet, 0.1–1.0 mL	—	1970001
Tips for Tensette Pipet	50/pkg	2185696

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Aluminum

DOC316.53.01002

Aluminon Method[1] **Method 8012**

(0.008 to 0.800 mg/L) **Powder Pillows**

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 36 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Digestion is required for determining total aluminum. Refer to the Digestion section of the Water Analysis Guide.
Clean all glassware with 6.0 N HCl and deionized water before use to remove contaminants from the glass.
Check the sample temperature. It must be between 20–25 °C (68 –77 °F) for accurate results.
The Pour-Thru Cell can be used if rinsed well with deionized water between the blank and prepared samples.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

Description	Quantity
AluVer® 3 Aluminum Reagent Powder Pillow	1
Ascorbic Acid Powder Pillow	1
Bleaching 3 Reagent Powder Pillow	1
50-mL graduated mixing cylinder with glass stopper	1
Sample cells (see *Instrument-specific information*)	2

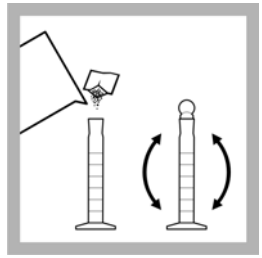
See *Consumables and replacement items* for reorder information.

Aluminon method with powder pillows

1. Select the test.

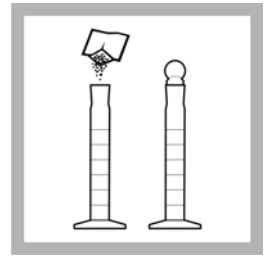
Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill the cylinder to the 50-mL mark with sample. Add one Ascorbic Acid Powder Pillow.

Stopper. Invert several times to dissolve the powder.

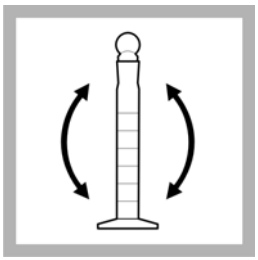


3. Add one AluVer 3 Aluminum Reagent Powder Pillow. Insert the stopper.

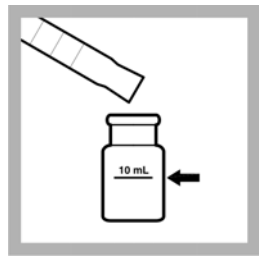
An orange to orange-red color will develop if aluminum is present.

4. Start the instrument timer.

A one-minute reaction period will begin.



5. Invert repeatedly for one minute to dissolve the powder. Undissolved powder will cause inconsistent results.



6. Blank Preparation: Pour 10 mL of the mixture into a square sample cell.

7. Add one Bleaching 3 Reagent Powder Pillow to the blank.

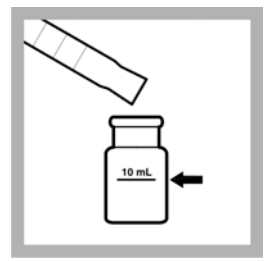
8. Start the instrument timer.

A 30-second reaction time will begin.

9. Vigorously swirl the cell for 30 seconds. The solution should turn a light to medium orange.

10. Start the instrument timer.

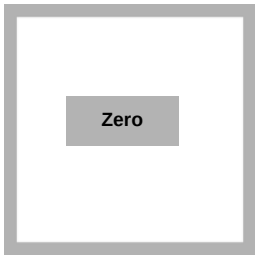
A 15-minute reaction period will begin.



11. Prepared Sample: Pour 10 mL of solution from the cylinder into a second square sample cell.

12. Within five minutes after the timer expires, wipe and dry the blank and place it into the cell holder.

Aluminon method with powder pillows (continued)

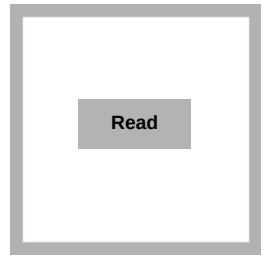


13. **ZERO** the instrument.

The display will show:

0.000 mg/L Al^{3+}

14. Immediately wipe and dry the prepared sample and place it into the cell holder.



15. **READ** the results in mg/L Al^{3+}.

See the user manual for instructions on changing the chemical form to Al_2O_3.

16. Clean the graduated cylinder with soap and brush immediately after the test. Rinse with deionized water.

Interferences

Table 37 Interfering substances

Interfering substance	Interference level
Acidity	Greater than 300 mg/L as $CaCO_3$. Samples with greater than 300 mg/L acidity as $CaCO_3$ must be treated as follows: **1.** Add one drop of m-Nitrophenol Indicator Solution[1] to the sample taken in step 2., before adding ascorbic acid. **2.** Add one drop of 5.0 N Sodium Hydroxide Standard Solution[1]. Stopper the cylinder. Invert to mix. Repeat as often as necessary until the color changes from colorless to yellow. **3.** Add one drop of 5.25 N Sulfuric Acid Standard Solution[1] to change the solution from yellow back to colorless. Continue with the test.
Alkalinity	1000 mg/L as $CaCO_3$. Interferences from higher alkalinity concentrations can be eliminated by the following pretreatment: **1.** Add one drop of m-Nitrophenol Indicator Solution[1] to the sample taken in step 2, before adding ascorbic acid. A yellow color indicates excessive alkalinity. **2.** Add one drop of 5.25 N Sulfuric Acid Standard Solution[1]. Stopper the cylinder. Invert to mix. If the yellow color persists, repeat until the sample becomes colorless. Continue with the test.
Fluoride	Interferes at all levels. See the *Fluoride interference graph*.
Iron	Greater than 20 mg/L
Phosphate	Greater than 50 mg/L
Polyphosphate	Polyphosphate interferes at all levels by causing negative errors and must not be present. Before running the test, polyphosphate must be converted to orthophosphate by acid hydrolysis as described under the phosphorus procedures.

[1] See *Optional reagents and apparatus* for reorder information.

Fluoride interferes at all levels by complexing with aluminum. The actual aluminum concentration can be determined using the *Fluoride interference graph* when the fluoride concentration is known.

To use the fluoride interference graph:

1. Select the vertical grid line along the top of the graph that represents the aluminum reading obtained in step 15.
2. Locate the point on the line where it intersects with the horizontal grid line that indicates how much fluoride is present in the sample.
3. Extrapolate the true aluminum concentration by following the curved lines on either side of the intersect point down to the true aluminum concentration.

For example, if the aluminum test result was 0.7 mg/L Al and the fluoride present in the sample was 1 mg/L F^-, the point where the 0.7 grid line intersects with the 1 mg/L F^- grid line falls between the 1.2 and 1.3 mg/L Al curves. In this case, the true aluminum content would be 1.27 mg/L.

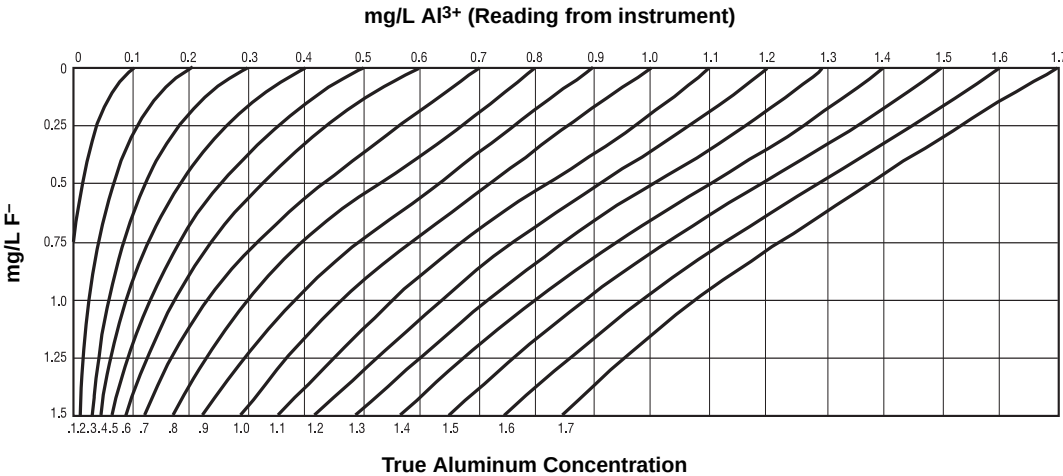


Figure 1 Fluoride interference graph

Sample collection, preservation and storage

Collect samples in clean glass or plastic containers. Preserve the sample by adjusting the pH to 2 or less with nitric acid (about 1.5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 3.5–4.5 with 5.0 N Sodium Hydroxide. Correct the test result for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Aluminum Voluette® Ampule Standard, 50-mg/L Al
- TenSette Pipet
- Mixing cylinders, (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Select Options>More>Standard additions from the instrument menu.
3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in the top row.
4. Open an Aluminum Voluette® Ampule Standard, 50-mg/L Al.
5. Prepare three sample spikes. Fill three mixing cylinders* with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view relationships between the sample spikes and the “Ideal Line” of 100% recovery.

Standard solution method

1. Prepare a 0.4-mg/L aluminum standard solution as follows: Pipet 1.00 mL of Aluminum Standard Solution, 100-mg/L as Al^{3+}, into a 250-mL volumetric flask.
2. Dilute to the mark with deionized water. Prepare this solution daily. Follow the *Aluminon method with powder pillows* test.

Or, use the following alternative procedure:

1. Using the TenSette Pipet, add 0.8 mL of solution from an Aluminum Voluette Ampule Standard Solution (50-mg/L as Al) into a 100-mL volumetric flask.
2. Dilute to volume with deionized water. Follow the *Aluminon method with powder pillows* test.
3. To adjust the calibration curve using the reading obtained with the 0.4-mg/L aluminum standard solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the shown concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

* See *Consumables and replacement items*.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
10	0.40 mg/L Al^{3+}	0.385–0.415 mg/L Al^{3+}	0.008 mg/L Al^{3+}

Summary of method

Aluminon indicator combines with aluminum in the sample to form a red-orange color. The intensity of color is proportional to the aluminum concentration. Ascorbic acid is added before the AluVer 3 reagent to remove iron interference. To establish a reagent blank, the sample is split after the addition of the AluVer 3. Bleaching 3 Reagent is then added to one-half of the split sample to bleach out the color of the aluminum aluminon complex. The AluVer 3 Aluminum reagent, packaged in powder form, shows exceptional stability and is applicable for fresh water applications. Test results are measured at 522 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Aluminum Reagent Set (100 Tests), includes:	—	—	2242000
(1) AluVer® 3 Aluminum Reagent Powder Pillow	1	100/pkg	1429099
(1) Ascorbic Acid Powder Pillow	1	100/pkg	1457799
(1) Bleaching 3 Reagent Powder Pillow	1	100/pkg	1429449
Hydrochloric Acid, 6.0 N	varied	500 mL	88449
Water, deionized	varied	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated mixing, 50-mL, with glass stopper	1	each	189641

Recommended standards

Description	Unit	Catalog number
Aluminum Standard Solution, 100-mg/L as Al^{3+}	100 mL	1417442
Aluminum Standard Solution, 10-mg/L as Al^{3+}	100 mL	2305842
Aluminum Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as Al	16/pkg	1479210
Voluette Ampule Breaker	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Liqui-Nox Phosphate-free detergent	946 mL	2088153
m-Nitrophenol Indicator Solution	100 mL	247632
Nitric Acid Solution, 1:1	500 mL	254049
pH Paper, 0–14 pH range	100/pkg	2601300
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips for 1970001	50/pkg	2185696
Sodium Hydroxide Standard Solution, 5.0 N	50 mL	245026
Sulfuric Acid Standard Solution, 5.25 N	100 mL	244932
Test Tube Brush	each	69000
Volumetric Flask, Class A, 100-mL	each	1457442
Volumetric Flask, Class A, 250-mL	each	1457446

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Edition 7

Aluminum

DOC316.53.01003

Eriochrome Cyanine R Method[1] **Method 8326**

(0.006 to 0.250 mg/L Al^{3+}) **Powder Pillows**

Scope and Application: For water

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 38 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

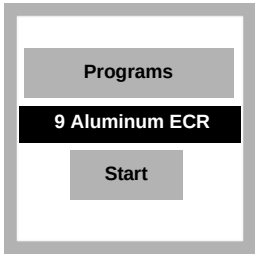
Clean all glassware with 6.0 N HCl and deionized water before use to remove contaminants from the glass.
Check the sample temperature. It must be between 20–25 °C (68 –77 °F) for accurate results.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

Description	Quantity
ECR Reagent Powder Pillow	1
ECR Masking Reagent Solution	1 drop
Hexamethylene-tetramine Buffer Reagent Powder Pillow	1
25-mL graduated mixing cylinder with glass stopper	1
Sample cells (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

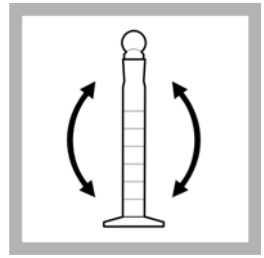
Eriochrome Cyanine R method with powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*). Refer to the user manual for orientation.

2. Fill the 25-mL mixing cylinder to the 20-mL mark with sample. Add one ECR Reagent Powder Pillow for 20-mL sample size.

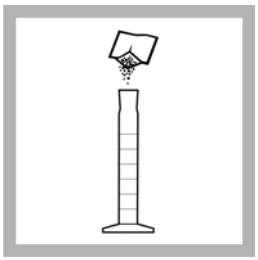


3. Insert the stopper. Invert several times to completely dissolve powder.

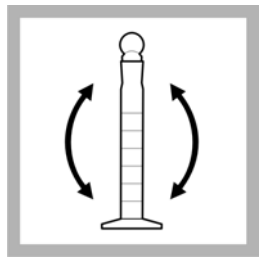
Undissolved reagent will cause inconsistent results.

4. Start the instrument timer.

A 30-second reaction period will begin.



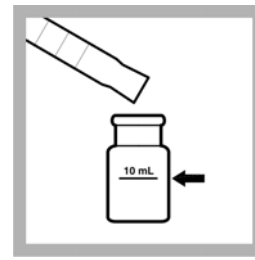
5. After the timer expires, add one Hexamethylene-tetramine Buffer Reagent powder pillow.



6. Insert the stopper. Invert several times to dissolve powder.

A red-orange color will develop if aluminum is present.

7. Blank Preparation: Put one drop of ECR Masking Reagent Solution into a clean square sample cell.



8. Pour 10 mL from the mixing cylinder into the blank cell. Swirl to mix.

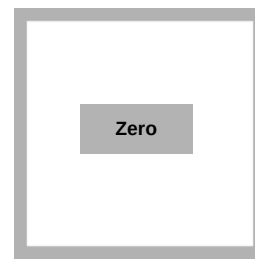
The solution will begin to turn yellow.

9. Prepared Sample: Fill a second square sample cell to the 10-mL mark with the remaining solution in the cylinder.

10. Start the instrument timer.

A 5-minute reaction period will begin.

11. Within five minutes after the timer expires, wipe the blank then insert it into the cell holder.



12. ZERO the instrument.

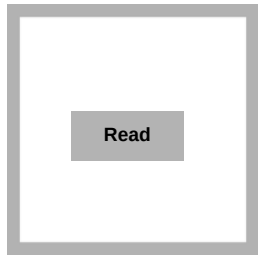
The display will show:

0.000 mg/L Al^{3+}

This test uses a non-zero intercept for the calibration curve on some instrument platforms.

Eriochrome Cyanine R method with powder pillows (continued)

13. Immediately wipe and dry the prepared sample then insert it into the cell holder.



14. Read the results in mg/L Al^{3+}.

See the user manual for instructions to change to alternate form Al_2O_3.

If fluoride is present, measure the fluoride concentration and refer to the *Fluoride Concentration (mg/L)* table.

Interferences

Table 39 Interfering substances

Interfering substance	Interference level and treatments
Acidity	Greater than 62 mg/L as $CaCO_3$
Alkalinity	Greater than 750 mg/L as $CaCO_3$
Ca^{2+}	Greater than 1000 mg/L as $CaCO_3$
Cl^-	Greater than 1000 mg/L as Cl^-
Cr^{6+}	0.2 mg/L (error is –5% of reading)
Cu^{2+}	2 mg/L (error is –5% of reading)
Fe^{2+}	Greater than 4 mg/L (error is positive and equals mg/L Fe^{2+} x 0.0075)
Fe^{3+}	Greater than 4 mg/L (error is positive and equals mg/L Fe^{2+} x 0.0075)
F^-	See the Fluoride Concentration (mg/L) table.
Hexameta phosphate	0.1 mg/L as PO_4^{3-} (error is –5% of reading)
Mg^{2+}	Greater than 1000 mg/L as $CaCO_3$
Mn^{2+}	Greater than 10 mg/L
NO_2^-	Greater than 5 mg/L
NO_3^-	Greater than 20 mg/L
pH	2.9–4.9 or 7.5–11.5. A sample pH between about 4.9 and 7.5 causes dissolved aluminum to partially convert to colloidal and insoluble forms. This method measures much of that hard-to-detect aluminum without any pH adjusting pretreatment as is necessary in some other methods.
PO_4^{3-} (ortho)	4 mg/L (error is –5% of reading)

Table 39 Interfering substances (continued)

Interfering substance	Interference level and treatments
Polyphosphate	See procedure below.
SO_4^{2-}	Greater than 1000 mg/L
Zn^{2+}	Greater than 10 mg/L

Polyphosphate interference can be reduced by converting polyphosphate to orthophosphate by the following steps:

1. Rinse a 50-mL graduated mixing cylinder and a 125-mL Erlenmeyer flask containing a magnetic stir bar with 6 N hydrochloric acid. Rinse again with deionized water. This will remove any aluminum present.

   Note: *Rinse two Erlenmeyer flasks if a reagent blank is used; see step 2.*

2. Measure 50 mL deionized water into the 125-mL Erlenmeyer flask using the graduated cylinder. This is the reagent blank. Because of the test sensitivity, this step must be done only when any of the reagents used in the following pretreatment are replaced—even if the new reagent has a matching lot number. When the pretreated sample has been analyzed, correct for the aluminum concentration of the reagent blank. Refer to the instrument user manual.
3. Measure 50 mL sample into the 125-mL Erlenmeyer flask using the graduated cylinder. Use a small amount of deionized water to rinse the cylinder contents into the flask.
4. Add 4.0 mL of 5.25 N Sulfuric Acid Standard Solution*.
5. Use a combination hot plate/stirrer to boil and stir the sample for at least 30 minutes. Add deionized water as needed to maintain a sample volume of 20-40 mL. Do not boil dry.
6. Cool the solution to near room temperature.
7. Add 2 drops of Bromphenol Blue Indicator Solution*.
8. Add 1.5 mL of 12.0 N Potassium Hydroxide Standard Solution* using the calibrated, plastic dropper provided. Swirl to mix. The solution color should be yellow or green but not purple. If the color is purple, begin with step 1 again using an additional 1 mL Sulfuric Acid Standard Solution in step 4.
9. While swirling the flask, add 1.0 N Potassium Hydroxide Solution*, a drop at a time, until the solution turns a dirty green color.
10. Pour the solution into the graduated cylinder. Rinse the flask contents into the graduated cylinder with deionized water to bring the total volume to 50 mL.
11. Use this solution in step 2. of the ECR method.

Note: *Fluoride interference can be corrected by using the Fluoride Concentration (mg/L) table.*

Example:

If the fluoride concentration is known to be 1.00 mg/L F^- and the ECR method gives a reading of 0.060 mg/L aluminum, what is the true mg/L aluminum concentration?

Intermediate values can be found by interpolation. Do not use correction graphs or charts found in other publications.

Answer: 0.183 mg/L

* See *Optional reagents and apparatus* for reorder information

Table 40 Fluoride Concentration (mg/L)

(mg/L)	0.00	0.20	0.40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.010	0.019	0.030	0.040	0.052	0.068	0.081	0.094	0.105	0.117	0.131
0.020	0.020	0.032	0.046	0.061	0.077	0.099	0.117	0.137	0.152	0.173	0.193
0.030	0.030	0.045	0.061	0.077	0.098	0.124	0.146	0.166	0.188	0.214	0.243
0.040	0.040	0.058	0.076	0.093	0.120	0.147	0.174	0.192	0.222	—	—
0.050	0.050	0.068	0.087	0.109	0.135	0.165	0.188	0.217	—	—	—
0.060	0.060	0.079	0.100	0.123	0.153	0.183	0.210	0.241	—	—	—
0.070	0.070	0.090	0.113	0.137	0.168	0.201	0.230	—	—	—	—
0.080	0.080	0.102	0.125	0.152	0.184	0.219	—	—	—	—	—
0.090	0.090	0.113	0.138	0.166	0.200	0.237	—	—	—	—	—
0.100	0.100	0.124	0.150	0.180	0.215	—	—	—	—	—	—
0.120	0.120	0.146	0.176	0.209	0.246	—	—	—	—	—	—
0.140	0.140	0.169	0.201	0.238	—	—	—	—	—	—	—
0.160	0.160	0.191	0.226	—	—	—	—	—	—	—	—
0.180	0.180	0.213	—	—	—	—	—	—	—	—	—
0.200	0.200	0.235	—	—	—	—	—	—	—	—	—
0.220	0.220	—	—	—	—	—	—	—	—	—	—
0.240	0.240	**True Aluminum Concentration (mg/L) Al**									

Sample collection, preservation and storage

Collect samples in clean glass or plastic containers. Preserve samples by adjusting the pH to 2 or less with concentrated nitric acid (about 1.5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 2.9–4.9 with 12.0 N Potassium Hydroxide Standard Solution* and/or 1 N Potassium Hydroxide Solution*. Correct the test result for volume additions.

Accuracy check

Required for accuracy check:

- Class A glassware (pipettes and volumetric flasks)
- Aluminum Standard Solution, 100 mg/L

  OR

- Aluminum Voluette® Ampule Standard Solution, 50-mg/L
- Deionized water

Standard solution method
Prepare a 0.100 mg/L aluminum standard solution as follows:

1. Using Class A glassware, pipet 1.00 mL of Aluminum Standard Solution 100 mg/L as Al^{3+}, into a 1000-mL volumetric flask.
2. Dilute to the mark with deionized water.

* See Optional reagents and apparatus for reorder information.

3. Prepare this solution daily. Do the *Eriochrome Cyanine R method with powder pillows*. Go to step 4.

OR

1. Add 2.0 mL of solution from an Aluminum Voluette Ampule Standard Solution (50-mg/L as Al) into a 1000-mL volumetric flask.
2. Dilute to volume with deionized water. Prepare this solution daily.
3. Do the *Eriochrome Cyanine R method with powder pillows* test procedure.
4. Select Options>More>Standard additions from the instrument menu.
5. Accept the shown concentration. If an alternate concentration is used, enter the actual concentration.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
9	0.100 mg/L Al^{3+}	0.091–1.009 mg/L Al^{3+}	0.002 mg/L Al^{3+}

Summary of method

Eriochrome Cyanine R combines with aluminum in a sample to produce an orange-red color. The intensity of color is proportional to the aluminum concentration. Test results are measured at 535 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Aluminum Reagent Set (100 Tests), includes:	—	—	2603700
ECR Reagent Powder Pillows	1	100/pkg	2603849
Hexamethylenetetramine Buffer Reagent Powder Pillows	1	100/pkg	2603999
ECR Masking Reagent Solution	1 drop	25 mL SCDB	2380123

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated mixing, 25-mL, with glass stopper	1	each	189640
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Thermometer, –10 to 110 °C	—	each	187701

Recommended standards

Description	Unit	Catalog number
Aluminum Standard Solution, 100-mg/L as Al^{3+}	100 mL	1417442

Recommended standards

Description	Unit	Catalog number
Aluminum Standard Solution, 10-mg/L as Al^{3+}	100 mL	2305842
Aluminum Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as Al	16/pkg	1479210
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule breaker for Voluette Standards	each	2196800
Bromphenol Blue Indicator Solution	100 mL	1455232
Cylinder, graduated mixing, 50-mL, Class A	each	2636341
Deionized Water	500 mL	27249
Erlenmeyer Flask, 125-mL	each	50543
Hot Plate with Stirrer, 7 x 7 inches, 115 VAC	each	2881600
Hydrochloric Acid, 6.0 N	500 mL	88449
Nitric Acid 1:1	500 mL	254049
pH Paper, 0–14 pH range	100/pkg	2601300
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips for 1970001	50/pkg	2185696
Potassium Hydroxide Standard Solution, 12.0 N	100 mL	23032
Potassium Hydroxide Solution, 1.0 N	50 mL	2314426
Sodium Hydroxide Standard Solution, 5.0 N	50 mL	245026
Stir Bar, magnetic, octagonal, 22.2 x 7.9 mm	each	2095350
Sulfuric Acid Standard Solution, 5.25 N	100 mL	244932
Volumetric Flask, Class A, 1000-mL	each	1457453

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Edition 7

Silver Diethyldithiocarbamate Method[1] Method 8013

(0 to 0.200 mg/L As)

Scope and Application: For water, wastewater, and seawater; distillation is required; USEPA accepted[2] for reporting for drinking and wastewater analysis (distillation required)

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

[2] Procedure is equivalent to Standard Method 3500-As for drinking water analysis.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding DR 3800, DR 2800, DR 2700information required to perform this test.

Table 41 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2612602	Fill line faces right
DR 5000	2612602	Fill line faces user
DR 3900	2612602	Fill line faces user
DR 3800, DR 2800, DR 2700	2612602	Fill line faces right

Before starting the test:

Create a user-entered program for arsenic. See step 1 and *User programming*.
Prepare the arsenic absorber solution as directed in *Reagent preparation*.
Perform a user-entered calibration for each new lot of arsenic absorber solution. See the *Calibration* section. Some variations of the calibration procedure are possible.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.
Do not use the Pour-Thru Cell with this test.
The arsenic absorber in this test is a silver solution in pyridine. Both silver (D011) and pyridine (D038) are regulated by the Federal RCRA as hazardous waste. In addition, the cotton ball soaked in lead acetate (D008) solution is a hazardous waste. These materials should not be poured down the drain. Refer to a current MSDS sheet for proper disposal.

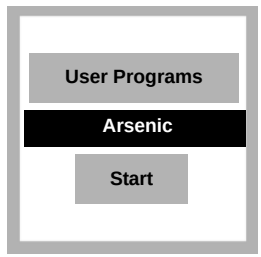
Collect the following items :

Description	Quantity
Apparatus (see *Required apparatus*)	—
Arsenic Standard Solution, 1000-mg/L As	varies
Hydrochloric Acid, ACS	25 mL
Lead Acetate Solution, 10%	1 mL
Potassium Iodide Solution, 20%	3 mL
Pyridine, ACS	50 mL
Sample Cells (see *Instrument-specific information*)	2
Silver Diethyldithiocarbamate	1 g
Stannous Chloride Solution	1 mL
Water, deionized	varies
Zinc, 20-mesh, ACS	6 g

See *Consumables and replacement items* for reorder information.

Silver Diethyldithiocarbamate

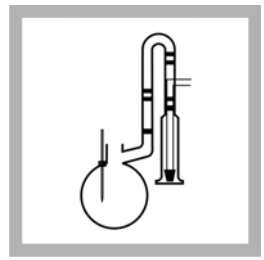
1. Perform the *User programming* procedure. Make note of the program number.



2. To run the test, press **USER PROGRAMS**.

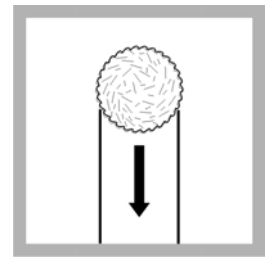
Select the test.

Insert an adapter if required (Table 1). Refer to the user manual for orientation.



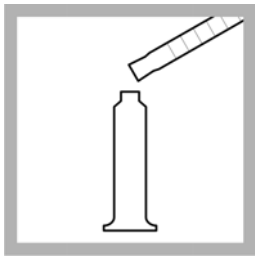
3. Prepare the distillation apparatus for arsenic recovery. See the *Distillation Manual* for assembly instructions. **Do not connect to the aspirator.**

Place the distillation apparatus under a fume hood to vent toxic fumes.



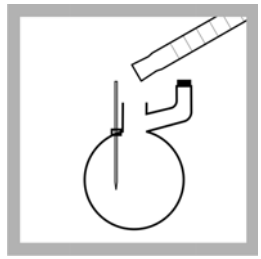
4. Dampen a cotton ball with 10% Lead Acetate Solution. Insert it in the gas scrubber. Be certain that the cotton seals against the glass.

Silver Diethyldithiocarbamate



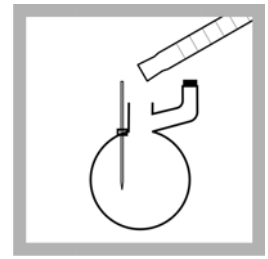
5. Using a graduated cylinder, pour 25-mL of prepared arsenic absorber solution (*Reagent preparation*) into the cylinder/gas bubbler assembly.

Attach it to the distillation apparatus.

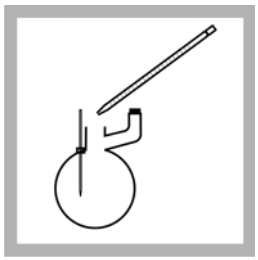


6. Using a graduated cylinder, pour 250 mL of sample into the distillation flask.

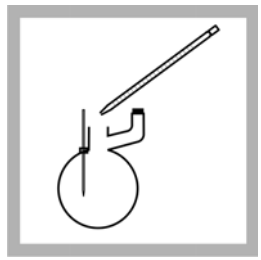
7. Turn on the power switch. Set the stir control to 5. Set the heat control to 0.



8. Using a graduated cylinder, add 25 mL of Hydrochloric Acid, ACS, to the distillation flask.



9. Use a serological pipet to add 1 mL of Stannous Chloride Solution to the flask.



10. Use a serological pipet to add 3 mL of Potassium Iodide Solution to the flask. Cap.

11. Start the instrument timer.

A 15-minute reaction period will begin.

12. When the timer expires, weigh and add 6.0 g of 20-mesh zinc to the flask. **Cap immediately.**

13. Set the heat control to 3.

14. Start the instrument timer.

A second 15-minute reaction period will begin.

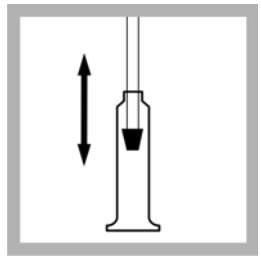
15. When the timer expires, set the heat control to 1.

16. Start the instrument timer.

A third 15-minute reaction period will begin.

Silver Diethyldithiocarbamate

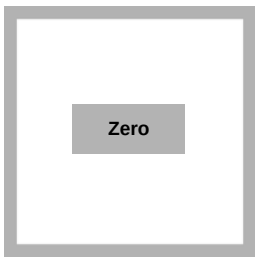
17. When the timer expires, turn off the heater. Remove the cylinder/gas bubbler assembly as a unit.



18. Rinse the gas bubbler by moving it up and down in the arsenic absorber solution.

19. Blank Preparation: Fill a dry, 10-mL sample cell with untreated arsenic absorber solution. Stopper.

20. Wipe the blank and insert it into the cell holder.



21. ZERO the instrument. The display will show the intercept as calculated from the user-entered calibration curve. This will probably be a non-zero intercept.

22. Prepared Sample: Pour the reacted arsenic absorber sample into a sample cell.

Close the sample cell.

23. Wipe the prepared sample and insert it into the cell holder.

READ the results.

Interferences

Table 42 Interfering substances

Interfering substance	Interference level
Antimony Salts	May interfere with color development.

Sample collection, preservation and storage

Collect samples in acid washed glass or plastic bottles. Adjust the pH to 2 or less with sulfuric acid (about 2 mL per liter)*. Preserved samples may be stored up to six months at room temperature. Correct the test result for volume additions.

* See *Optional reagents and apparatus*.

Reagent preparation

Prepare the arsenic absorber solution as follows:

1. Weigh 1.00 g of silver diethyldithiocarbamate on an analytical balance.
2. Transfer the powder to a 200-mL volumetric flask. Dilute to volume with pyridine. **Use pyridine only in a fume hood and wear chemical resistant gloves.** Read the MSDS before using pyridine.
3. Mix well to dissolve. Store the reagent, tightly sealed, in an amber bottle. The reagent is stable for one month if stored in this manner. Larger volumes of reagent can be prepared if the reagent is used within one month.

Calibration

Standard preparation

Perform a new calibration for each lot of arsenic absorber solution.

1. Prepare a 10.0-mg/L arsenic working standard by pipetting 10.0 mL of Arsenic Standard Solution, 1000 mg/L As into a 1000-mL volumetric flask.
2. Dilute to volume with deionized water.
3. Into three different 500-mL volumetric flasks, pipet 1.0, 2.0, and 10.0 mL of the 10.0 mg/L As stock solution using Class A glassware.
4. Dilute to the mark with deionized water and mix thoroughly. These standards have concentrations of 0.02, 0.04 and 0.20 mg/L As.

   Note: *Distill standards before making the calibration curve.*

User programming

1. Press **USER PROGRAMS** on the main menu.
2. Press **PROGRAM OPTIONS** and **NEW**. Key any available program number (950–999) to use for arsenic testing. Press **OK**.
3. Fill in the appropriate fields using the touch screen when the field is highlighted. Use the alphanumeric keys to name the User Program **ARSENIC**. Press **NEXT** to move to the next screen. Set up the rest of the parameters as follows:

   - •Program Type: Single Wavelength
   - •Units: mg/L
   - •Concentration Resolution: 0.001
   - •Chemical Form: As
   - •Wavelength: 520 nm
   - •Calibration: Read Standards

4. After entering Read Standards, press **NEXT>EXIT**. Fill in the appropriate fields for each of the following. Use the touch screen to activate the parameter and press **EDIT** to enter the data entry screen. Set up the rest of the parameters as follows:

   - •Timer1: 15 minutes
   - •Timer2: 15 minutes
   - •Timer3: 15 minutes
   - •Upper Limit: 0.220 mg/L
   - •Lower Limit: –0.020 mg/L

5. Press **CALIBRATION: C = A + BA**. Press **EDIT**.

6. The Read Standards will be indicated. Enter the standard concentration values to be used to perform the calibration: 0.00, 0.02, 0.04, and 0.20. To enter the concentration values press **+** and enter the value followed by **OK** for each concentration value.
7. After the values are entered, press the **UP** arrow four times to move the cursor to the 0.00 concentration line.
8. Insert the 25-mL sample cell containing only unreacted arsenic absorber solution into the cell holder. Press **ZERO.**
9. Press the **DOWN** arrow once to move to the next concentration. Insert the prepared sample in the cell holder. Press **READ** to accept the absorbance value. Repeat steps for each standard.

   Note: *Standards must be distilled before absorbance values are measured.*

10. Press **GRAPH**. Make sure **FORCE ZERO** is off.
11. If the graph is acceptable press **DONE>EXIT**.
12. “Store Program?” will appear on the display. Press **YES**.

The program is ready for use.

Some variations of the calibration procedure are possible. See the user manual for details.

Summary of method

Arsenic is reduced to arsine gas by a mixture of zinc, stannous chloride, potassium iodide, and hydrochloric acid in a specially equipped distillation apparatus. The arsine is passed through a scrubber containing cotton saturated with lead acetate for sulfide removal, and then into an absorber tube containing silver diethyldithiocarbamate in pyridine. The arsenic reacts to form a red complex which is read colorimetrically. This procedure requires a manual calibration. Test results are measured at 520 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Arsenic Standard Solution, 1000-mg/L As	varies	100 mL	1457142
Hydrochloric Acid, ACS	25 mL	500 mL	13449
Lead Acetate Solution, 10%	1 mL	100 mL	1458042
Potassium Iodide Solution, 20%	3 mL	100 mL	1456842
Pyridine, ACS	50 mL	500 mL	1446949
Silver Diethyldithiocarbamate	1 g	25 g	1447624
Stannous Chloride Solution	1 mL	100 mL	1456942
Water, deionized	varies	4 liters	27256
Zinc, 20-mesh, ACS	6 g	454 g	79501

Required apparatus

Description	Quantity	Unit	Catalog number
Balance, analytical, Zeta series, 80-g capacity	1	each	2936701
Balls, cotton	1	100/pkg	257201
Boat, weighing, 8.9-cm square	2	500/pkg	2179000

Required apparatus (continued)

Description	Quantity	Unit	Catalog number
Bottle, amber, 237-mL, glass	1	6/pkg	714441
Cap, polypropylene, for amber bottle	1	6/pkg	2166706
Cylinder, graduated, 25-mL	2	each	50840
Cylinder, graduated, 250-mL	1	each	50846
Distillation Apparatus, arsenic accessories	1	set	2265400
Distillation Apparatus, general purpose accessories	1	set	2265300
Flask, volumetric, Class A, 1000-mL, with glass stopper	1	each	1457453
Flask, volumetric, Class A, 200-mL	1	each	1457445
Flask, volumetric, Class A, 500-mL	6	each	1457449
Pipet Filler, safety bulb	1	each	1465100
Pipet, serological, 5-mL	2	each	53237
Pipet, volumetric, Class A, 1.00-mL	2	each	1451535
Pipet, volumetric, Class A, 2.00-mL	1	each	1451536
Pipet, volumetric, Class A, 4.00-mL	1	each	1451504
Pipet, volumetric, Class A, 6.00-mL	1	each	1451506
Pipet, volumetric, Class A, 8.00-mL	1	each	1451508
Pipet, volumetric, Class A, 10.00-mL	1	each	1451538
Select one based on available voltage:			
Distillation Apparatus Heater, 115 VAC, 60 Hz	1	each	2274400
Distillation Apparatus Heater, 230 VAC, 50 Hz	1	each	2274402

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25-mL	each	189640
Sulfuric Acid, 1.00 N	100 mL	127032
Gloves, chemical resistant, size 9[1]	pair	2410104

[1] Other sizes available.

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Edition 7

Atrazine

DOC316.53.01006

Immunoassay[1] **Method 10050**

Scope and Application: For water

[1] This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 43 Instrument-specific information

Instrument	Adapter
DR 6000	—
DR 5000	A23618
DR 3900	LZV846(A)
DR 3800, DR 2800, DR 2700	LZV583

Before starting the test:

This method analyzes for Atrazine in water. Sample calibrators and reagents are added to cuvettes coated with Atrazine-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Read the entire procedure before starting. Identify and make ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.

Timing is critical; follow instructions carefully.

A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.

Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.

Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.

To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.

The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the spectrophotometer.

Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.

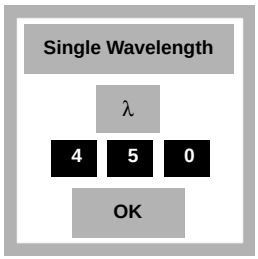
Protective nitrile gloves are recommended for this procedure.

Collect the following items:

Description	Quantity
Atrazine Reagent Set	1
Caps, flip spout	1
Marker, laboratory	1
Rack, for 1-cm Micro Cuvettes	1
Wipes, disposable	1
Pipet, TenSette®, 0.1–1.0 mL	1
Pipet Tips, for TenSette Pipet 19700-01	1

See *Consumables and replacement items* for reorder information.

Atrazine immunoassay method



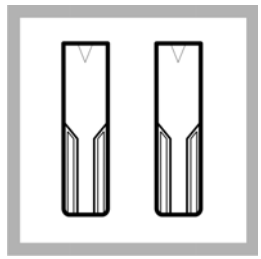
1. Press

Single Wavelength

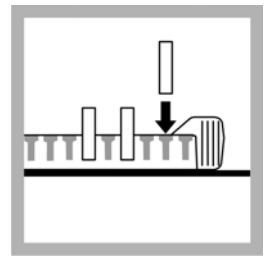
Press **OPTIONS** and the λ button.

Enter **450 nm** and press **OK.**

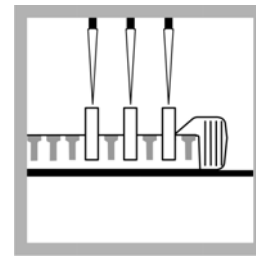
Insert an adapter if required (see *Instrument-specific information*). Refer to the user manual for orientation.



2. Label an Antibody Cuvette for each calibrator and each sample to be tested.



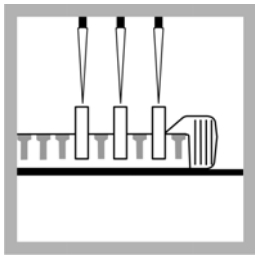
3. Insert the cuvettes into the rack snugly.



4. Pipet 0.5 mL of each **calibrator** into the appropriately labeled cuvette.

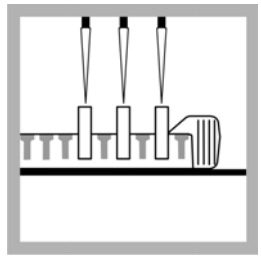
Use a new pipette tip for each calibrator.

Atrazine immunoassay method (continued)



5. Pipet 0.5 mL of each **sample** to be tested into the appropriately labeled cuvette.

Use a new pipette tip for each sample.



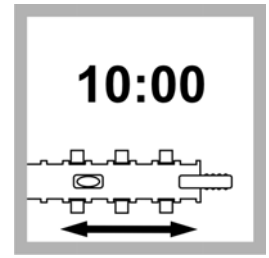
6. Immediately pipet 0.5 mL of Atrazine Enzyme Conjugate into each cuvette.

Use the same pipette tip for each sample.

7. Press **OPTIONS**. Press **TIMER**. Enter 20:00 minutes and press **OK.**

A 20-minute reaction time will begin.

Immediately mix the contents of the cuvettes for 30 seconds (see *Using the 1-cm MicroCuvette Rack*.)



8. After 10 minutes mix the contents of the rack for 30 seconds (see *Using the 1-cm MicroCuvette Rack*.)

9. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

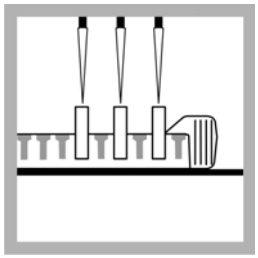
10. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Ensure that most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.

Atrazine immunoassay method (continued)

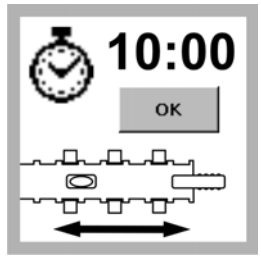
Color Development

Important Note: *Timing is critical. Follow instructions carefully.*



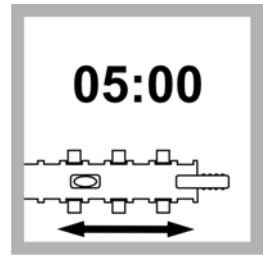
11. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Use the same pipette tip for each sample.



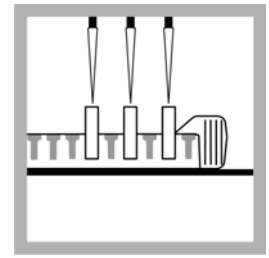
12. Set the instrument timer for 10:00 minutes Press **OK** to start a 10-minute reaction time.

Mix, using the instructions is *Using the 1-cm MicroCuvette Rack*.



13. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Solutions will turn blue in some or all of the cuvettes.

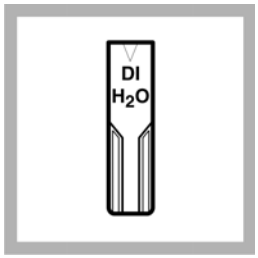


14. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 11. Use the same pipette tip repeatedly for this step.

Slide the rack for 20 seconds (see *Using the 1-cm MicroCuvette Rack*.)

Blue solutions will turn yellow with the addition of the Stop Solution.

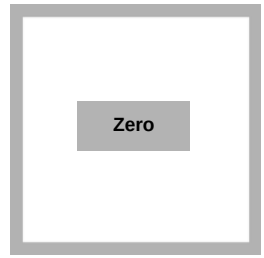
Atrazine immunoassay method (continued)



15. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

16. Insert the filled Zeroing Cuvette into the cell holder.

Orient the arrow in the same direction for all cuvettes.



17. **ZERO** the instrument.

The display will show:

0.000 Abs

18. Insert the first calibrator into the cell holder.

READ the results. The display will give an absorbance reading. Record the results for each calibrator and sample.

Repeat this step for all remaining calibrators and samples.

See *Interpreting and reporting results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

The 1-cm MicroCuvette Rack has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

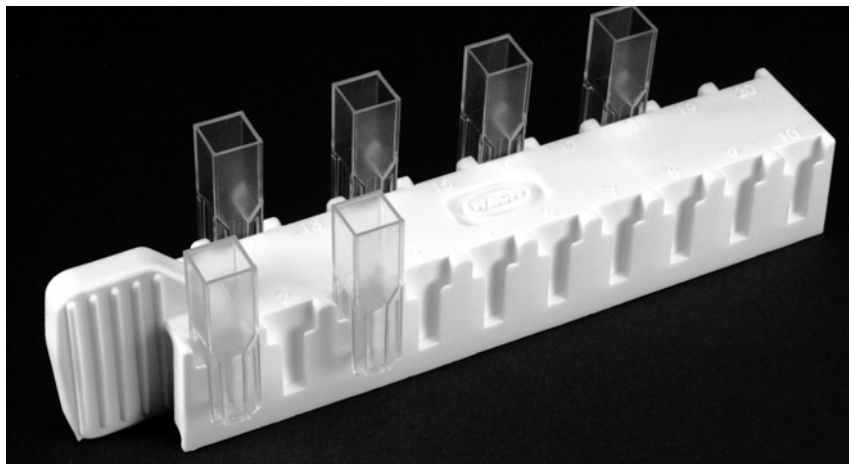


Figure 1 The 1-cm MicroCuvette Rack

Loading the Rack—The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and insert all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing—Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and reporting results

There is an inverse relationship between the concentration of Atrazine and the absorbance reading. In other words, the higher the reading, the lower the concentration of Atrazine. See the *Relative Atrazine concentration* table.

Table 44 Relative Atrazine concentration

If the sample reading is...	the sample Atrazine Concentration is...
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example

Readings:
0.5 ppb Atrazine Calibrator: **0.475 Abs**

3.0 ppb Atrazine Calibrator: **0.245 Abs**

Sample #1: **0.140 Abs**

Sample #2: **0.300 Abs**

Sample #3: **0.550 Abs**

Interpretation
Sample #1—Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Atrazine is greater than both 0.5 ppb and 3.0 ppb Atrazine.

Sample #2—Sample reading is between the readings for the 0.5 ppb and 3.0 ppb Atrazine calibrators. Therefore the sample concentration of Atrazine is between 0.5 ppb and 3.0 ppb.

Sample #3—Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Atrazine is less than both 3.0 ppb and 0.5 ppb.

Storing and Handling Reagents

1. Wear protective gloves and eyewear.
2. When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
3. Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
4. If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Atrazine immunoassay test cannot differentiate between certain triazines and metabolites, but it detects their presence to differing degrees. The *Required concentrations for selected chemicals*

table shows the required concentration for selected chemicals. The *Compounds tested but not detectable up to 10,000 ppb* table shows compounds not detectable at 10,000 ppb.

Table 45 Required concentrations for selected chemicals

Compound	Concentration to give a positive result at 3 ppb (in ppb)
Ametryne	1
Atrazine	3
Atrazine, de-ethylated	115
Atrazine, de-isopropyl	714
Cyanazine	460
Cyromazine	1200
Prometon	8
Prometryne	0.7
Propazine	2.3
Simetryne	5.4
Simazine	37
Terbuthylazine	91
Terbutryne	8.3

Table 46 Compounds tested but not detectable up to 10,000 ppb

Alachlor	2, 4-D
Aldicarb	Diaminoatrazine
Carbendazim	Melamine
Carbofuran	Metolachlor

Sample collection and storage

- Collect samples in a clean glass bottle.
- **Do not pre-rinse the bottle with the sample.**
- If the sample cannot be analyzed immediately, store the sample at 4 °C.
- Samples may be kept for as long as 14 days.
- Warm stored samples to room temperature before analysis.

Water sample dilution

Other levels of Atrazine can be tested by diluting the sample and comparing the results to the 0.1 ppb Calibrator. Select the appropriate sample volume from the *Sample volume and concentration* table, place it in a graduated mixing cylinder, and dilute it to 50 mL with deionized water.

Table 47 Sample volume and concentration

mL sample	Representative concentration using 0.1 ppb calibrator
0.5	10 ppb
1.0	5 ppb
2.5	2 ppb
5.0	1 ppb

Example:
Dilute 2.5 mL of sample to 50 mL with deionized water. Run the diluted sample in the procedure along with the 0.1 ppb calibrator. If the absorbance of the diluted sample is less than the 0.1 ppb calibrator, the concentration of the original sample is greater than 2 ppb.

Summary of method

Immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Atrazine-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Atrazine from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Atrazine compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Atrazine and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Atrazine in the sample. The resulting color is then compared with a calibrator to determine whether the Atrazine concentration in the sample is greater or less than the threshold levels. Test results are measured at 450 nm.

Consumables and replacement items

Required reagents

Description	Unit	Cat. No.
Atrazine Reagent Set[1]	20 cuvettes	2762700

[1] Immunoassay components are manufactured by Beacon Analytical Systems, Inc.

Required apparatus

Description	Unit	Cat. No.
Caps, flip spout	2/pkg	2581802
Marker, laboratory	each	2092000
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Rack, for 1-cm Micro Cuvettes	each	4879900
Wipes, disposable	box	2097000

Optional reagents and apparatus

Description	Unit	Cat. No.
Atrazine Reagent Set	100 cuvettes	2762710
Glasses, safety	each	2756800
Gloves, disposable, nitrile, medium[1]	100/pkg	2550502
Graduated Mixing Cylinder, 50-mL	each	2636341
Graduated Mixing Cylinder, 25-mL	each	2636340
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Water, deionized	500 mL	27249

[1] Other sizes are available.

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Edition 7

Barium

DOC316.53.01007

Turbidimetric Method[1] **Method 8014**

(2 to 100 mg/L) **Powder Pillows**

Scope and Application: For water, wastewater, oil-field water, and seawater

[1] Adapted from Snell and Snell, *Colorimetric Methods of Analysis, Vol. II*, 769 (1959).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 48 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Perform a standard curve adjustment or a new calibration for each new lot of reagent. See *Standard solutions* and *Calibration standard preparation*.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Filter highly colored or turbid water samples using a funnel[1] and filter paper[1]. Large amounts of color or turbidity will interfere and cause high readings.
If samples cannot be analyzed immediately, refer to *Sample collection, preservation and storage*. Adjust the pH of preserved samples before analysis.
The Pour-Thru and Sipper Cell modules cannot be used with this procedure.

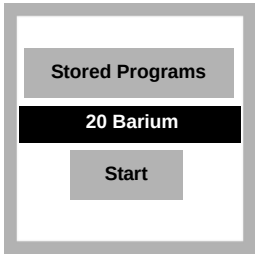
[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
BariVer® 4 Barium Reagent Powder Pillows	1
Sample cells (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

Turbidimetric method with powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Fill a square sample cell with 10 mL of sample.

3. Prepared Sample: Add the contents of one BariVer® 4 Barium Reagent Powder Pillow to the cell. Swirl to mix.

If barium is present, a white turbidity will develop.

If the reagent does not dissolve readily in the sample, mix the sample and reagent in a 25-mL graduated mixing cylinder before pouring into the sample cell.

4. Start the instrument timer.

A five-minute reaction period will begin.

Do not disturb the sample during the reaction period.

5. Blank Preparation: Fill another square sample cell with 10 mL of sample.

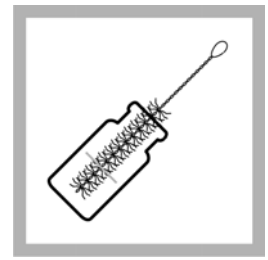
6. When the timer expires, wipe the blank and insert the blank into the cell holder.

ZERO the instrument. The display will show:

0 mg/L Ba^{2+}

7. Wipe and the prepared sample and insert the prepared sample into the cell holder.

READ the results in mg/L Ba^{2+}.



8. Clean the sample cell immediately after each test. Use soap, water and a brush to prevent a film of barium sulfate from forming inside the cell.

Interferences

Table 49 Interfering substances

Interfering substance	Interference levels and treatments
Calcium	10,000 mg/L as $CaCO_3$
Magnesium	100,000 mg/L as $CaCO_3$

Table 49 Interfering substances (continued)

Interfering substance	Interference levels and treatments
Silica	500 mg/L
Sodium Chloride	130,000 mg/L as NaCl
Strontium	Interferes at any level. If present, the total concentration between barium and strontium may be expressed as a PS (Precipitated by Sulfate). While this does not distinguish between barium and strontium, it gives an accurate indication of scaling tendency.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

Collect samples in an acid cleaned glass or plastic container. Adjust the pH to 2 or less with nitric acid* (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 5 with 5.0 N sodium hydroxide*. Correct the test result for volume additions.

Standard solutions

Prepare a 90.0-mg/L barium standard solution as follows:

1. Pipet 9.00 mL of Barium Standard Solution, 1000-mg/L, into a 100-mL volumetric flask.
2. Dilute to the mark with deionized water.
3. Prepare this solution daily. Follow the *Turbidimetric method with powder pillows* procedure.
4. To adjust the calibration curve using the reading obtained with the standard solution, select Options>More>Standard Adjust from the instrument menu.
5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Accuracy check

Required for accuracy check:

- Barium Standard Solution, 1000-mg/L Ba
- Pipet, TenSette®, 0.1–1.0 mL and tips

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Select Options>More>Standard additions from the instrument menu.
3. Default values for standard concentration, sample volume, and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Barium Standard Solution, 1000-mg/L Ba.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer expires, read the result. Press **READ** to accept the reading.

* See *Optional reagents and apparatus*.

6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Touch the timer icon. After the timer expires, read the result. Press **READ** to accept the reading.
7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Touch the timer icon. After the timer expires, read the result. Press **READ** to accept the reading.
8. Each addition should reflect approximately 100% recovery.
9. After completing the sequence, press GRAPH to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press IDEAL LINE to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

Calibration standard preparation

Prepare calibration standards containing 10, 20, 30, 50, 80, 90, and 100 mg/L Ba as follows:

1. Into seven different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 5, 8, 9, and 10 mL of the 1000-mg/L Barium Standard Solution using Class A glassware.
2. Dilute to the mark with deionized water. Mix thoroughly.
3. Using the turbidimetric method and the calibration procedure described in the user manual, generate a calibration curve from the standards prepared above.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
20	DR 5000	30 mg/L Ba	25–35 mg/L Ba	1 mg/L Ba

Summary of method

The BariVer® 4 Barium Reagent Powder combines with barium to form a barium sulfate precipitate, which is held in suspension by a protective colloid. The amount of turbidity present caused by the fine white dispersion of particles is directly proportional to the amount of barium present. Test results are measured at 450 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
BariVer® 4 Barium Reagent Powder Pillows	1	100/pkg	1206499

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Barium Standard Solution, 1000-mg/L Ba	100 mL	1461142
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Filter Paper for Funnel, 12.5 cm	100/pkg	189457
Funnel, 65 mm	each	108367
Liqui-Nox Phosphate-free Detergent	946 mL	2088153
Nitric Acid 1:1	500 mL	254049
pH Paper, 0–14 pH range	100/pkg	2601300
Pipet, TenSette, 0.01–1.0 mL	each	1970001
Pipet Tips for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips for TenSette Pipet 1970001	1000/pkg	2185628
Sodium Hydroxide, 5.0 N	100 mL	245032
Test Tube Brush	each	69000
Volumetric Flask, Class A, 100-mL	each	1457442

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Edition 7

Benzotriazole/Tolyltriazole

DOC316.53.01008

UV Photolysis Method[1]

Method 8079

(Benzotriazole 1.0 to 16.0 mg/L)
(Tolyltriazole 1.0 to 20.0 mg/L)

Powder Pillows

Scope and Application: For cooling or boiler water.

[1] Adapted from Harp, D., *Proceedings 45th International Water Conference*, 299 (October 22–24, 1984)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 50 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

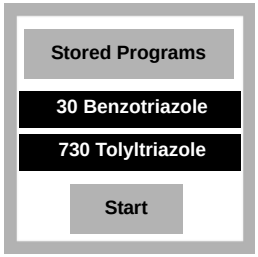
Avoid fingerprints on the quartz surface of the lamp. Rinse the lamp and wipe with a soft, clean tissue between tests.
Check the sample temperature. It must be between 20–25 °C (68 –78 °F) for accurate results.
If sample contains nitrite or borax (sodium borate), adjust the pH to 4–6 with 1 N sulfuric acid.
If the sample contains more than 500 mg/L hardness (as $CaCO_3$), add 10 drops of Rochelle Salt Solution before adding reagent.

Collect the following items:

Description	Quantity
Triazole Reagent powder pillows	1
Sample cells (see *Instrument-specific information*)	2
Square bottle, 25-mL	1
Ultra-violet lamp with power supply	1
UV safety goggles	1

See *Consumables and replacement items* for reorder information.

UV Photolysis method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Prepared Sample: Fill a marked mixing bottle to the 25 mL line with sample.

3. Add the contents of one Triazole Reagent Powder Pillow.

Swirl to dissolve completely.

4. Put on UV safety goggles.

5. Insert the ultraviolet lamp into the mixing bottle.

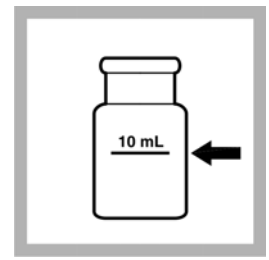
Turn on the UV lamp.

6. Start the instrument timer. A 5-minute reaction period will begin.

Low results will occur if photolysis (lamp on) takes place for more or less than five minutes.

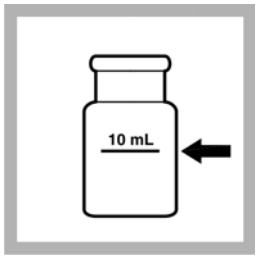
A yellow color will form if triazole is present.

7. When the timer expires, turn the lamp off. Remove the lamp from the bottle. Swirl the bottle to mix thoroughly.



8. Fill a square sample cell with 10 mL of reacted (prepared) sample.

UV Photolysis method for powder pillows (continued)



9. Blank Preparation:

Fill another square sample cell with 10 mL of sample.

10. Insert the blank into the cell holder.

ZERO the instrument.

The display will show:

0.0 mg/L Benzotriazole

or

0.0 mg/L Tolyltriazole

11. Insert the prepared sample into the cell holder.

READ the results in mg/L Benzotriazole or mg/L Tolyltriazole.

Interferences

Table 51 Interfering substances

Interfering substance	Interference level
Acrylates (as methyl acrylate)	Greater than 50 mg/L
Alum	Greater than 400 mg/L
Borate (as sodium tetraborate)	Greater than 4000 mg/L. Adjust the pH to 4–6 with 1 N sulfuric acid[1].
Chlorine (as Cl_2)	Greater than 20 mg/L
Chromium (as chromate)	Greater than 12 mg/L
Copper	Greater than 10 mg/L
Hardness	Greater than 500 mg/L as $CaCO_3$. Add 10 drops of Rochelle Salt Solution[1] before adding reagent.
Iron	Greater than 20 mg/L
Lignosulfonates	Greater than 40 mg/L
Magnesium	Greater than 300 mg/L as $CaCO_3$
Molybdenum (as molybdate)	Greater than 200 mg/L
Nitrite	Greater than 4000 mg/L. Adjust the pH to 4–6 with 1 N sulfuric acid[1].
Phosphonates (AMP or HEDP)	Greater than 100 mg/L
Sulfate	Greater than 200 mg/L
Zinc	Greater than 80 mg/L
Strong oxidizing or reducing agents	Interfere at all levels

[1] See *Consumables and replacement items*.

Sample collection, preservation and storage

The most reliable results are obtained when samples are analyzed as soon as possible after collection.

Accuracy check

Required for accuracy check:

- Benzotriazole Standard Solution, 500 mg/L
- Pipet, TenSette®, 0.1–1.0 mL and tips

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard additions from the instrument menu.
3. Default values for standard concentration, sample volume, and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a 500-mg/L Benzotriazole Standard Solution*.
5. Prepare three sample spikes. Fill three mixing cylinders with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Follow the *UV Photolysis method for powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Accept each standard addition result by pressing READ. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, select **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Select **IDEAL LINE** to view relationships between the sample spikes and the “Ideal Line” of 100% recovery.

UV Lamp Check
To verify the ultraviolet lamp (normal life equals 5000 hours) is working properly, perform the following test:

1. Prepare a 5.0 mg/L benzotriazole standard solution by pipetting 10.0 mL of Benzotriazole Standard Solution, 500-mg/L benzotriazole, into a 1-liter volumetric flask. Dilute to volume.
2. Analyze according to the above procedure. If the result is significantly below 5 mg/L, replace the lamp.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
30	DR 5000	10 mg/L benzotriazole	9.7–10.3 benzotriazole	0.2 mg/L benzotriazole
730	DR 5000	12 mg/L tolyltriazole	11.6–12.4 mg/L tolyltriazole	0.2 mg/L tolyltriazole

* See *Optional reagents and apparatus*.

Summary of method

Benzotriazole or tolyltriazole, used in many applications as corrosion inhibitors for copper and copper alloys, are determined by a proprietary catalytic ultraviolet (UV) photolysis procedure requiring less than 10 minutes to perform. Test results are measured at 425 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Triazole Reagent Powder Pillows	1	100/pkg	2141299

Required apparatus

Description	Quantity	Unit	Catalog number
UV Safety Goggles	1	each	2113400
Bottle, Square, with 25 mL mark	1	each	1704200
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Select one based on available voltage:			
Lamp Kit, UV, with power supply, 115 VAC, 60 Hz	1	each	2704500
Lamp Kit, UV, with power supply, 230 VAC, 50 Hz	1	each	2704502

Recommended standards

Description	Unit	Catalog number
Benzotriazole Standard Solution, 500-mg/L	100 mL	2141342

Optional reagents and apparatus

Description	Unit	Catalog number
Flask, Volumetric, Class A, 1000 mL	each	1457453
pH Paper, 0–14 pH range	100/pkg	2601300
Pipet Bulb	each	1465100
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips for TenSette Pipet 1970001	1000/pkg	2185628
Sulfuric Acid, 1 N	100 mL	127032
Rochelle Salt Solution	29 mL	172533
Volumetric pipet, Class A, 10-mL	each	1451538

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Edition 7

Boron

DOC316.53.01009

Carmine Method[1] **Method 8015**

(0.2 to 14.0 mg/L) **Powder Pillows**

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 52 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

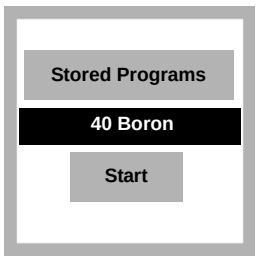
All labware must be completely dry. Excess water will cause low results.
Use the BoroVer® 3 Reagent with adequate ventilation. See *Reagent preparation*.
Do not cap the sample cells or the Erlenmeyer flasks at any time during sample preparation or reaction time. Samples may be capped immediately prior to placing in the instrument.
Sulfuric acid may contain residual moisture; this will cause low results. Make sure the sulfuric acid is suitable by running a known boron standard before running any unknown samples.
Wear safety goggles and gloves during this test.

Collect the following items:

Description	Quantity
BoroVer 3 Reagent Powder Pillow	1
Cylinders, graduated, 50-mL and 100 mL	1 of each
Flask, Erlenmeyer, 125-mL	2
Flask, Erlenmeyer, 250-mL	1
Pipet Bulb	1
Pipet, volumetric, 2.0 mL	2
Sulfuric Acid, Concentrated	75 mL
Sample Cells (see *Instrument-specific information*)	2
Water, deionized	2.0 mL

See *Consumables and replacement items* for reorder information.

Carmine method with powder pillows

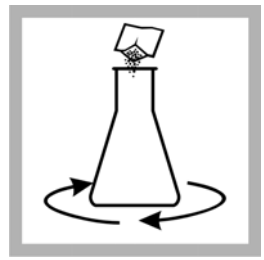


1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

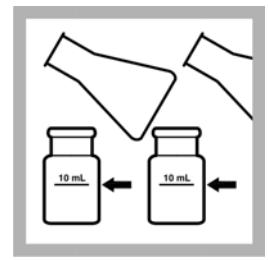
Refer to the user manual for orientation.

2. Using a 100-mL graduated cylinder, measure 75 mL of concentrated sulfuric acid. Pour the acid into a 250-mL Erlenmeyer flask.



3. With good ventilation, add the contents of one BoroVer 3 Reagent Powder Pillow to the flask. Swirl to mix. Allow up to five minutes for the powder to dissolve completely.

4. Blank Preparation: Accurately pipet 2.0 mL of deionized water into a 125-mL Erlenmeyer flask.



Carmine method with powder pillows (continued)

5. Prepared Sample: Accurately pipet 2.0 mL of sample into another 125-mL Erlenmeyer flask.

6. Using a 50-mL graduated cylinder, add 35 mL of the solution prepared in step 3 to each Erlenmeyer flask.

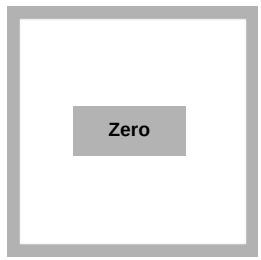
Swirl to mix completely.

7. Start the instrument timer.

A 25-minute reaction period will begin.

8. When the timer expires, pour at least 10 mL from each flask into separate square sample cells.

9. Wipe the blank and insert the blank into the cell holder.

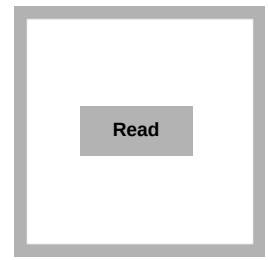


10. ZERO the instrument.

The display will show:

0.0 mg/L B

11. Wipe and insert the prepared sample into the cell holder.



12. READ the results in mg/L B.

Sample collection, preservation and storage

Collect samples in clean polyethylene or polypropylene bottles, or alkali-resistant, boron-free glass.

Reagent preparation

To prepare additional BoroVer 3/Sulfuric Acid Solution, mix one BoroVer 3 Reagent Powder Pillow per 75 mL of concentrated sulfuric acid, adding the powder pillows individually while stirring. Preparation of this solution generates gaseous HCl when the indicator pillow is added to the sulfuric acid. Use of a fume hood or other well-ventilated lab area is strongly advised. This solution is stable for up to 48 hours if stored in plastic containers. **Do not store in borosilicate glassware (Pyrex® or Kimax®) for longer than one hour;** the solution may leach boron from these containers.

The BoroVer 3/Sulfuric Acid Solution is highly acidic. Refer to the current MSDS for safe handling and disposal instructions.

Accuracy check

Required for accuracy check:

- Boron Standard Solution, 1000-mg/L as B
- Pipet Bulb
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Volumetric pipet, 5-mL
- Volumetric pipet, 15-mL
- Mixing cylinder, 25-mL

Standard additions method (sample spike)

1. Prepare a 250 mg/L boron standard as follows:

a. Pipet 5.00 mL of Boron Standard Solution, 1000 mg/L as B into a mixing cylinder.

b. Pipet 15.00 mL of demineralized water into the cylinder, stopper and mix thoroughly.

2. After reading test results, leave the unspiked sample in the instrument. Verify the chemical form.

3. Select Options>More>Standard Additions from the instrument menu.

4. Default values for standard concentration, sample volume, and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.

5. Prepare three sample spikes. Fill three mixing cylinders* with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.

6. Follow the *Carmine method with powder pillows* test. Analyze 2.0 mL of each sample spike starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solution Method

Required for accuracy check:

- Boron Standard Solution, 1000-mg/L B
- Volumetric flask, 200-mL
- Volumetric pipet, Class A and bulb
- Deionized water

1. Prepare a Boron Standard Solution, 10-mg/L as B. solution as follows:

   a. Use Class A glassware to pipet 2.00 mL of the Boron Standard Solution, 1000-mg/L B, into a 200-mL volumetric flask.

   b. Dilute to volume with deionized water.

   c. Swirl to mix. Follow the Boron test procedure.

2. To adjust the calibration curve using the reading obtained with the standard solution, select Options>More>Standard Adjust from the instrument menu.

3. Turn on the Standard Adjust feature and accept the shown concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

* See *Optional reagents and apparatus*.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
40	7.6 mg/L B	7.5–7.7 mg/L B	0.14 mg/L B (at 0.2 mg/L)
			0.16 mg/L B (at 7.0 mg/L)
			0.18 mg/L B (at 14.0 mg/L)

Summary of Method

Boron is determined by its reaction with carminic acid in the presence of sulfuric acid to produce a reddish to bluish color. The amount of color is directly proportional to the boron concentration. Test results are measured at 605 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
BoroVer® 3 Boron Reagent Powder Pillows	1	100/pkg	1417099
Sulfuric Acid, ACS, concentrated	75 mL	2.5 L	97909
Water, deionized	2.0 mL	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Flask, Erlenmeyer, 125-mL	2	each	50543
Flask, Erlenmeyer, 250-mL	1	each	50546
Pipet, volumetric, 2.0-mL, Class A	2	each	1451536
Safety Bulb	1	each	1465100
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Boron Standard Solution, 1000 mg/L as B	100 mL	191442

Optional reagents and apparatus

Description	Unit	Catalog number.
Cylinder, mixing, 25 mL	each	2088640
Gloves, chemical resistant, size 9 to 9.5	pair	2410104[1]
pH Paper, 0–14 pH range	100/pkg	2601300
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for 1970001	50/pkg	2185696
Pipet Tips, for 1970001	1000/pkg	2185628
Pipet, volumetric, 5.00 mL, Class A	each	1451537
Pipet, volumetric, 15.00 mL, Class A	each	1451539
Safety Goggles, vented	each	2550700
Volumetric Flask, Class A, 200-mL	each	1457445

[1] Other sizes available

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Edition 7

Boron

DOC316.53.01010

Azomethine-H Method[1] **Method Number**

LR (0.02 to 1.50 mg/L as B) **Powder Pillows**

Scope and Application: For testing low levels of boron (boric acid or borates) in drinking water, cooling water, industrial process waters or wastewaters.

[1] Adapted from ISO Method 9390 Harp, D.L., Analytica Chimica Acta, 346(1997), pp. 373–379.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 53 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2410212	Fill line faces right
DR 5000	2410212	Fill line faces user
DR 3900	2410212	Fill line faces user
DR 3800, DR 2800, DR 2700	2410212	Fill line faces right

Before starting the test:

For best results, match two cells using the *Cell matching procedure*.
Sample temperature should be 22–24 °C (72–75 °F) for most accurate results.
If outside this range, measure and record the sample temperature. See *Sample temperature compensation*.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

Collect the following items:

Description	Quantity
BoroTrace™ Reagent Set Includes:	—
EDTA Solution, 1M	20 drops
BoroTrace™ #2 Reagent Powder Pillow	2
BoroTrace™ #3 Reagent Powder Pillow	2
Water, Ultra-pure, aldehyde-free	25 mL
Sample cell (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

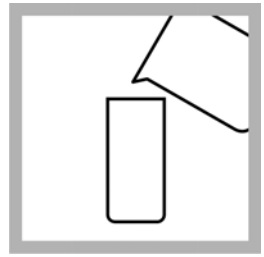
Azomethine-H method

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

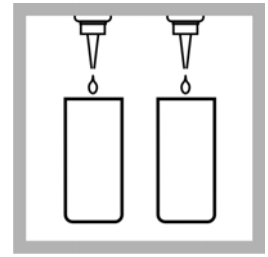
Refer to the user manual for orientation.

2. Blank Preparation: Fill a clean plastic sample cell to the 25-mL mark with **ultra-pure** water.



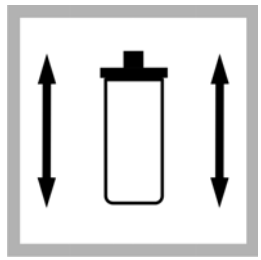
3. Prepared Sample: Fill a second clean plastic sample cell to the 25-mL mark with sample.

If the sample is colored or turbid, sample pretreatment may be required See the *Interfering substances and suggested treatments* table.



4. Add ten drops of EDTA Solution, 1 M, to each cell. Cap and invert each cell twice to mix.

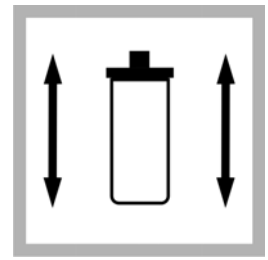
5. Open one BoroTrace™ 2 Reagent powder pillow and add the contents to the prepared sample.



6. Cap the prepared sample and shake to dissolve the powder. Proceed immediately with steps 7 through 10.

7. Start the instrument timer.

A ten-minute reaction period will begin.

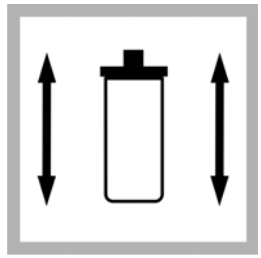


8. Continue shaking vigorously for 30 seconds, or until all powder is dissolved

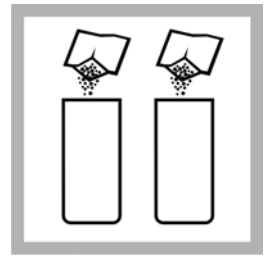
Let the cell sit capped for the remaining reaction period.

Azomethine-H method (continued)

9. During the reaction period, add the contents of a BoroTrace™ 2 Reagent Powder Pillow to the blank.



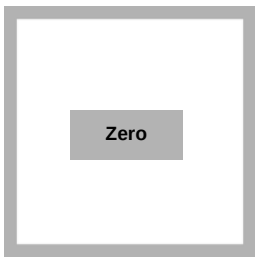
10. Cap the blank and shake vigorously until the powder is dissolved.



11. After the timer expires, add the contents of one BoroTrace™ 3 Reagent Powder Pillow to each cell. Cap and shake to dissolve.

BoroTrace™ 3 Reagent "stops" the reaction.

12. Wipe the blank and insert it into the cell holder.

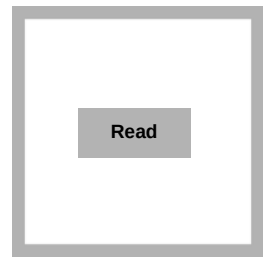


13. **ZERO** the instrument.

The display will show:

0.00 mg/L B

14. Wipe the prepared sample and insert it into the cell holder.



15. **READ** the results in mg/L B.

To correct for sample temperature, refer to the *Sample temperature compensation* table. To change to alternate form H_3BO_3, refer to the instrument user manual.

Cell matching procedure

1. Rinse and fill two cells with deionized water.
2. Wipe each cell with a soft cloth or tissue to remove liquid or fingerprints.
3. Using one of the cells, set the instrument absorbance at 410 nm to zero.
4. Read the absorbance of the other cell.
5. Cells that read within 0.002 absorbance are matched.

Interferences

The substances listed in the *Interfering substances* table have been tested for interference and found not to interfere up to the indicated levels (in mg/L). The *Interfering substances and suggested treatments* table lists suggested treatments for interferences:

Table 54 Interfering substances

Interfering substance	Interference level
Aluminum ($^{3+}$)	10
Benzotriazole	20
Biocides:	
Carbamate-type	120
Isothiazolin-type	120
Quat-type	90
Thiocyanate-type	60
Calcium	1000 (as $CaCO_3$)
Chloride	2500
Copper ($^{2+}$)	20
Magnesium	1000 (as $CaCO_3$)
Manganese ($^{7+}$)	5
Molybdate (Mo^{6+})	60
Phosphonates, AMP	20
Phosphonates, HEDP	20
Polyacrylates	20 (as Acumer 1000, 1100)
Polymaleic Acid	40 (as Belclene 200)
Silica	120
Sulfate	1800
Sulfite	40
Tolyltriazole	20
Zinc ($^{2+}$)	10

Table 55 Interfering substances and suggested treatments

Interfering substance	Interference level and treatments
Alkalinity >500 mg/L (+ or –)	**1.** Adjust sample pH to between 5–7 using 1.0 N Sulfuric Acid Solution[1]. **2.** Continue with step 4 of the *Azomethine-H method* procedure.
Color (+)	**1.** Zero the instrument (0.00 mg/L B) using Ultra-Pure Water[2]. Measure and record the apparent concentration, in mg/L B, due to the sample color. **2.** Subtract the apparent concentration from the result in step 15 of the *Azomethine-H method* procedure.
Halogens (Bromine or Chlorine) all levels (+)	Halogen disinfectants in the sample can produce a red-color after the addition of BoroTrace™ 2 Reagent. To eliminate this interference: **1.** Add 1 pillow Dechlorinating Reagent[1] to 25 mL each of Ultra-Pure Water[2] and sample. **2.** Cap and shake to dissolve. **3.** Continue with step 4 of the test procedure.

Table 55 Interfering substances and suggested treatments (continued)

Interfering substance	Interference level and treatments
Iron (Fe3+ or Fe2+), above 8 mg/L (+)	High levels of iron in the sample can produce a red-color after the addition of BoroTrace™ 2 Reagent. To compensate, increase the amount of EDTA[2] from 10 drops to 15 drops to be added to each cell (step 4). Alternatively, dilute the sample with Ultra-Pure Water and continue with step 5 of the test procedure. Correct the results (step 15) using the appropriate dilution factor.
Nitrites, all levels (+)	**1.** Add 0.1 gram scoop Sulfamic Acid[1] to 25 mL each Ultra-Pure Water and sample in plastic cells. **2.** Cap and shake to dissolve. **3.** Uncap and wait 5 minutes. **4.** Add 5 N Sodium Hydroxide Reagent[1] solution to each to adjust pH to 5–8 using pH paper. **5.** Continue with step 4 of the test procedure.
Turbidity (+)	Filter the sample through a 3 µm membrane1 prior to testing. Do not use a glass fiber filter.

[1] See *Optional reagents and apparatus*.

[2] See *Required reagents*.

Sample collection, preservation and storage

Collect samples in clean polyethylene bottles. Do not use borate-based detergents or soaps to clean sample containers or labware used for this method. After use, thoroughly rinse all plastic containers with deionized water, allow to air dry, and keep covered.

Sample temperature compensation

The reaction chemistry is very dependent on the sample temperature. Calibrations are performed at 23 °C (73 °F). If the sample temperature is outside the range of 22–24 °C (72–75 °F), multiply the results, in mg/L, by the appropriate multiplier (see the *Sample temperature multipliers* table).

Table 56 Sample temperature multipliers

Sample Temp.		Multiplier	Sample Temp.		Multiplier
°C	°F		°C	°F	
5	41	0.70	20	68	0.94
7	45	0.73	25	77	1.04
10	10	0.78	26	79	1.06
12	54	0.81	27	81	1.08
14	57	0.84	28	82	1.10
16	61	0.87	29	84	1.12
18	64	0.91	30	86	1.15

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- 1000-mg/L Boron Standard Solution
- 100-mL plastic volumetric flask with stopper
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Deionized water

- Volumetric pipet, 5-mL
- Pipet bulb

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume, and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare a 50.0-mg/L boron standard:
   a. Pipet 5.0 mL of a 1000-mg/L Boron Standard Solution into a 100-mL plastic volumetric flask.
   b. Dilute with deionized water.
   c. Insert a stopper and invert to mix.
5. Prepare three sample spikes. Fill three mixing cylinders* with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of the diluted standard, respectively, to each sample and mix thoroughly.
6. Follow the *Azomethine-H method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Required for accuracy check:

- Boron Standard Solution, 1000 mg/L as B
- Deionized water
- Plastic volumetric flask, 1000-mL
- Plastic pipet

1. Prepare a 1.0-mg/L B standard:
   a. Use a plastic pipet to transfer 1.0 mL of Boron Standard Solution, 1000 mg/L as B, into a 1000-mL plastic volumetric flask.
   b. Dilute to volume with deionized water.
   c. Insert a stopper and invert to mix.
2. To adjust the calibration curve using the reading obtained with the standard solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

* See *Optional reagents and apparatus*

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
45	0.92 mg/L B^{3+}	0.87–0.97 mg/L B	0.01 mg/L B

Summary of method

Azomethine-H, a Schiff base, is formed by the condensation of an aminonaphthol with an aldehyde by the catalytic action of boron. Test results are measured at 410 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
BoroTrace™ Reagent Set, includes:	—	—	2666900
EDTA Solution, 1 M	20 drops	50 mL SCDB	2241926
BoroTrace™ #2 Reagent Powder Pillows	2	100/pkg	2666669
BoroTrace™ #3 Reagent Powder Pillows	2	100/pkg	2666799
Water, Ultra-pure, Aldehyde-free	25-mL	500 mL	2594649

Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
Sample cell, 1-inch square plastic, with cap	2	12/pkg	2410212

Recommended standards

Description	Unit	Catalog number
Boron Standard Solution, 1000-mg/L as B	100 mL	191442

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25 mL	each	2088640
Dechlorinating Reagent, Powder Pillows	100/pkg	1436369
Filter Holder, 25 mm	each	246800
Membrane, filter, 3 µm, 25 mm	25/pkg	2594025
pH Paper, 1.0–11.0	5 rolls/pkg	39133
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet, tips for 1970001 TenSette Pipet	50/pkg	2185696
Pipet, volumetric, Class A, 5.00-mL	each	1451537
Pipet, volumetric, Class A, 1- mL	each	1451537
Safety Bulb	each	1465100
Sodium Hydroxide Reagent, 5.0 N, 50 mL	each	245026

Optional reagents and apparatus

Description	Unit	Catalog number
Spoon, measuring, 0.1 g	each	51100
Sulfamic Acid, 454 g	each	234401
Sulfuric Acid Solution 1.0N, 100 mL	each	127032
Syringe, 30 cc Luer-lock	each	2225800
Thermometer, non-mercury, –10 to +225 °C	each	1451535
Volumetric Flask, Class A 1000-mL	each	1451535

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Edition 7

Bromine

DOC316.53.01011

DPD Method[1] **Method 8016**

(0.05 to 4.50 mg/L) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For testing bromine residuals (including hypobromite, hypobromous acid and bromamines) used as disinfectants in process waters, treated water, estuary water, and seawater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 57 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

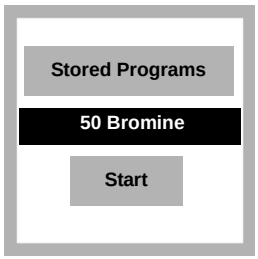
Analyze samples immediately. Do not preserve for later analysis.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
If the sample temporarily turns yellow after reagent addition, dilute a fresh sample and repeat the test. A slight loss of bromine may occur because of the dilution. Multiply the result by the dilution factor.
Sample cell orientation may vary. Refer to the instrument user manual for correct cell orientation.
Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
DPD Total Chlorine Reagent Powder Pillows	1
Sample cells (see *Instrument-specific information*)	2
AccuVac Test:	
DPD Total Chlorine Reagent AccuVac® Ampuls	1
Beaker, 50-mL	1
Sample cell (see *Instrument-specific information*)	1
Stopper, 18 mm tube	1

See Consumables and replacement items on page 183 for reorder information.

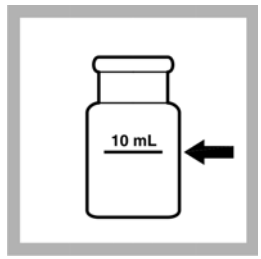
DPD Powder Pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a sample cell with 10 mL of sample.

3. Prepared Sample: Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell.

Swirl for 20 seconds to mix.

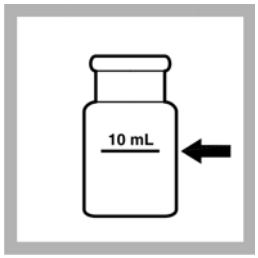
A pink color will develop if bromine is present.

4. Start the instrument timer.

A three-minute reaction period will begin.

Perform steps 5–6 during the reaction period.

DPD Powder Pillows (continued)



5. Blank Preparation: Fill a second sample cell with 10 mL of sample.

6. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

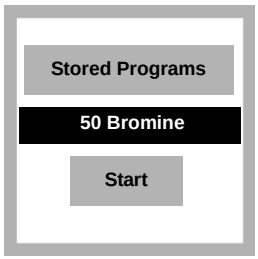
The display will show:

0.00 mg/L Br_2

7. Within three minutes after the timer expires, wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L Br_2.

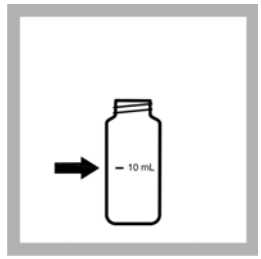
DPD AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a round 1-inch sample cell with 10 mL of sample.

3. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill a DPD Total Chlorine Reagent AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

4. Quickly invert the Ampul several times to mix.

A pink color will develop if bromine is present.

DPD AccuVac® Ampuls (continued)

5. Start the instrument timer.

A three-minute reaction period will begin.

Perform step 6 during the reaction period.

6. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show:

0.00 mg/L Br_2

7. Within three minutes after the timer expires, wipe the Ampul and insert it into the cell holder.

READ the results in mg/L Br_2.

Interferences

Table 58 Interfering substances

Interfering substance	Interference level
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Chlorine	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as $CaCO_3$
Iodine	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide[1] (30-g/L) to a 25-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops Sodium Arsenite[1, 2] (5-g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result of this test from the original analysis to obtain the correct bromine concentration.
Monochloramine	Interferes at all levels
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7.

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004).

Sample collection, preservation and storage

Collect samples in clean, dry glass containers. If sampling from a tap, allow the water to flow at least 5 minutes to ensure a representative sample. Avoid excessive agitation and exposure to sunlight when sampling. Allow several volumes of water to overflow the container and cap the container so there is no headspace above the sample. If sampling with a DR cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Proceed with the analysis immediately.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
50	2.80 mg/L Br_2	2.75–2.85 mg/L Br_2	0.05 mg/L Br_2
55	2.80 mg/L Br_2	2.71—2.89 mg/L Br_2	0.05 mg/L Br_2

Summary of method

Bromine residuals react with iodide and DPD (N,N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total bromine concentration. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
DPD Total Chlorine Reagent Powder Pillows	1	100/pkg	2105669
OR			
DPD Total Chlorine Reagent AccuVac® Ampuls	1	25/pkg	2503025

Required apparatus

Description	Quantity	Unit	Catalog number
AccuVac snapper	1	each	2405200
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
PourRite Ampule Breaker, 2-mL	each	2484600
Voluette Ampule Breaker, 10-mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
AccuVac Snapper	each	2405200
Chlorine Demand-Free Water	500 mL	2641549
Cylinder, mixing, 25 mL	each	2088640
Cylinder, mixing, 50 mL	each	189641
DPD Total Chlorine Reagent Powder Pillows	1000/pkg	2105628
DPD Total Chlorine Reagent Powder Pillows	300/pkg	2105603
pH Paper,0 –14 pH range	100/pkg	2601300
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sodium Hydroxide, 1 N	100 mL	104532
Sulfuric Acid, 1 N	100 mL	127032
Stopper for 18 mm tube	6/pkg	173106

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Edition 7

Cadmium

DOC316.53.01012

Dithizone Method[1] **Method 8017**

(0.7 to 80.0 µg/L) **Powder Pillows**

Scope and Application: For water and wastewater; digestion is required to determine total cadmium.

[1] Adapted from Standard Methods for the Examination of Water and Wastewater (method 3500 Cd D).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 59 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.
Clean all glassware with 6 N Hydrochloric Acid Solution and rinse with deionized water.
Sample cell orientation may vary. Refer to the instrument user manual for correct cell orientation.
Cloudy and turbid samples may require filtering before running the test. Report results as µg/L soluble cadmium. Use glass membrane type filter to avoid loss of cadmium by adsorption onto the filter paper.
If samples cannot be analyzed immediately, see *Sample collection, preservation and storage*. Adjust the pH of preserved samples before analysis.
The Flow Cell and Sipper Modules cannot be used with this procedure.
The DithiVer powder will not completely dissolve in the chloroform. For further notes see *Dithiver solution preparation and storage*.
Read the MSDS before testing. Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Goggles and gloves are recommended.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

Collect the following items:

Description	Quantity
Citrate buffer powder pillows	1
Chloroform	30 mL
DithiVer Metals Reagent powder pillows	1
Potassium Cyanide	0.1 g
Sodium Hydroxide solution, 50%	20 mL
Cotton balls	1
Clippers	1
Cylinder, 25-mL graduated	1
Cylinder, 250-mL graduated	1
Cylinder, 50-mL graduated mixing	1
Funnel, 500-mL separatory	1
Sample Cells	2
Spoon, measuring, 0.1-g	1
Support ring (4-inch) and stand (5 x 8-inch base)	1

See *Consumables and replacement items* for reorder information.

Dithizone method with powder pillows

DANGER
Cyanide is a deadly poison. Use a fume hood. Maintain cyanide solutions at pH 11 or greater to prevent formation of cyanide gas.

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Fill a 250-mL graduated cylinder to the 250-mL mark with sample. Pour the sample into a 500-mL separatory funnel.

3. Add the contents of one Buffer Powder Pillow for heavy metals, citrate type. Stopper the funnel and shake to dissolve.

4. DithiVer Solution Preparation:
Add 30 mL of chloroform to a 50-mL mixing graduated cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow. Stopper the cylinder. Invert several times to mix.

Dithizone method with powder pillows (continued)

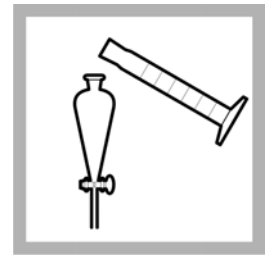
5. Add 20 mL of 50% Sodium Hydroxide Solution to the funnel.

6. Add a 0.1-g scoop of potassium cyanide to the funnel. Stopper. Shake vigorously for 15 seconds.

7. Remove the stopper.

Start the instrument timer.

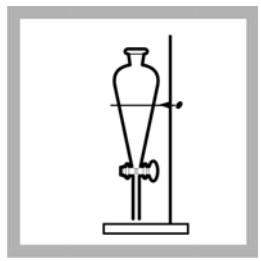
A 1-minute reaction period will begin.



8. Add 30 mL of the DithiVer solution to the 500-mL separatory funnel. Stopper, invert, and open stopcock to vent. Close the stopcock and shake funnel once or twice; vent again.

9. Start the instrument timer.

Close the stopcock and shake the funnel vigorously during the 1-minute time period.

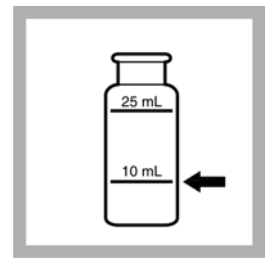


10. Start the instrument timer and allow the funnel to stand undisturbed until the timer expires.

The bottom (chloroform) layer will be orange to pink if cadmium is present.

11. Prepared Sample: Insert a cotton plug the size of a pea into the delivery tube of the funnel and slowly drain the bottom (chloroform) layer into a dry 25-mL sample cell (the prepared sample). Stopper.

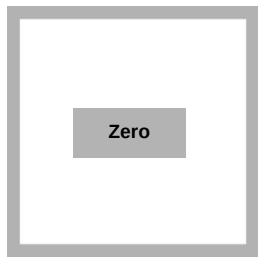
The cadmium-dithizone complex is stable for more than one hour if the sample cell is kept tightly capped and out of direct sunlight.



12. Blank Preparation: Fill a dry sample cell with at least 10 mL of chloroform. Stopper.

Dithizone method with powder pillows (continued)

13. Insert the blank into the cell holder.

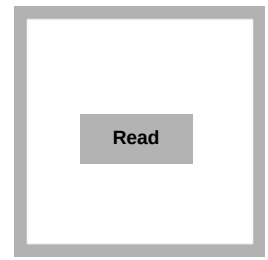


14. **ZERO** the instrument.

The display will show:

0.0 µg/L Cd

15. Insert the prepared sample into the cell holder.



16. **READ** the results in µg/L cadmium.

Interferences

Table 60 Interfering substances

Interfering substance	Interference level
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.
Bismuth	Greater than 80 mg/L. See treatment below.
Copper	Greater than 2 mg/L. See treatment below.
Mercury	All levels. See treatment below.
Silver	Greater than 2 mg/L. See treatment below.

To eliminate interference from the metals listed in the *Interfering substances* table, insert the following steps into the procedure after step 4.

1. Measure approximately 5 mL of the DithiVer solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red, or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals. Draw off and collect the bottom (chloroform) layer for proper disposal.
2. Repeat the extraction with fresh 5-mL portions of the DithiVer solution (discarding the bottom layer each time) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated several times without appreciably affecting the amount of cadmium in the sample.
3. Extract the solution with several 2- or 3-mL portions of pure chloroform to remove any remaining DithiVer, collecting the bottom layer each time for proper disposal.
4. Continue with step 5 of the procedure.
5. In step 8, substitute 28.5 mL of DithiVer solution for the 30 mL.
6. Continue with step 9 of the procedure.

Table 61 Substances that do not interfere

Aluminum	Lead
Antimony	Magnesium
Arsenic	Manganese
Calcium	Nickel
Chromium	Tin
Cobalt	Zinc
Iron	

Sample collection, preservation and storage

Collect samples in an acid-washed glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Store preserved samples up to six months at room temperature. Adjust the pH to 2.5 with 5.0 N sodium hydroxide before analysis. Correct the test result for volume additions.

Dithiver solution preparation and storage

Store DithiVer Powder Pillows away from light and heat. A convenient way to prepare this solution is to add the contents of 16 DithiVer Metals Reagent Powder Pillows to a 500-mL bottle of chloroform and invert several times until well mixed (carrier powder may not dissolve completely). Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.

Accuracy check

Required for accuracy check:

- Cadmium Voluette Ampule Standard, 25-mg/L Cd
- TenSette® Pipet, 0.1–1.0 mL and tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume, and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to three 250-mL samples and mix each thoroughly.
6. Follow the *Dithizone method with powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard Solution Method
Required for accuracy check:

- Cadmium Standard Solution, 100-mg/L Cd.
- Deionized water
- 100-mL Volumetric flask

- Class A volumetric pipet and bulb

1. Prepare a 5.0-mg/L cadmium standard solution:
   a. Pipet 5.00 mL of Cadmium Standard Solution, 100-mg/L Cd, into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Prepare this solution daily.
2. Pipet 2.00 mL of the 5.0-mg/L Cadmium Standard Solution into 248 mL of deionized water in a 500-mL separatory funnel. This is a 40-µg/L cadmium solution. Perform *Dithizone method with powder pillows* on this solution beginning with step 3 of the procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, elect Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the shown concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
60	40.0 µg/L Cd	39.3–40.7 µg/L Cd	0.73 µg/L

Summary of method

The dithizone method is designed for the determination of cadmium in water and wastewater. The DithiVer Metals Reagent is a stable powder form of dithizone. Cadmium ions in basic solution react with dithizone to form a pink to red cadmium-dithizonate complex, which is extracted with chloroform. Test results are measured at 515 nm.

Pollution prevention and waste management

Both chloroform (D022) and cyanide (D003) solutions are regulated as hazardous wastes by the Federal RCRA. Do not pour these solutions down the drain. Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent waste. Collect the cyanide solution as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent potential release of hydrogen cyanide gas. See the current MSDS for disposal information.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Cadmium Reagent Set	—	100/pkg	2242200
Includes:(1) 14202-99, (1) 14458-17, (1) 12616-99, (1) 767-14, (4) 2180-49, (1) 2572-01			
Buffer Powder Pillows, citrate	1	100/pkg	1420299
Chloroform, ACS	40 mL	4 L	1445817
DithiVer Metals Reagent Powder Pillows	1	100/pkg	1261699
Potassium Cyanide	0.1 g	125 g	76714
Sodium Hydroxide Solution, 50%	20 mL	500 mL	218049

Required reagents (continued)

Description	Quantity/Test	Unit	Catalog number
Cotton Balls, absorbent	1	100/pkg	257201

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers, for opening powder pillows	1	each	96800
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 250-mL	1	each	50846
Cylinder, graduated, mixing, 50-mL	1	each	189641
Funnel, separatory, 500-mL	1	each	52049
Sample cell, 25 mL square, matched pair with cap	2	2/pkg	2612602
Spoon, measuring, 0.1-g	1	each	51100
Support Ring, 4"	1	each	58001
Support Ring Stand, 5" x 8" base	1	each	56300

Recommended standards

Description	Unit	Catalog number
Cadmium Standard Solution, 100-mg/L Cd	100 mL	1402442
Chloroform, ACS	500 mL	1445849
Hydrochloric Acid Solution, 6.0 N	500 mL	88449
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Sodium Hydroxide Standard Solution, 5.0 N	59 mL SCDB	245026
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, graduated, 5-mL	each	50837
Filter Discs, glass, 47 mm	100/pkg	253000
Filter Holder, glass, for 47-mm filter	each	234000
Flask, Erlenmeyer, 500-mL	each	50549
Flask, filtering, 500-mL	each	54649
Flask, volumetric, Class A, 100-mL	each	1457442
Flask, volumetric, Class A, 250-mL	each	1457446
Flask, volumetric, Class A, 1000-mL	each	1457453
Gloves, chemical resistant, size 9 to 9.5[1]	pair	2410104
Goggles, safety, vented	each	2550700
Hot Plate, 3½-in. diameter, 120 VAC, 50/60 Hz	each	1206701
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	39133
Pipet Filler, safety bulb	each	1465100
Pipet, serological, 2-mL	each	53236
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, 2.00-mL, Class A	each	1451536
Pipet, volumetric, 3.00-mL, Class A	each	1451503
Pipet, volumetric, 6.00-mL, Class A	each	1451506
Pipet, volumetric, 8.00-mL, Class A	each	1451508
Pipet, volumetric, 9.00-mL, Class A	each	1451509
Pipet, volumetric, 10.00-mL, Class A	each	1451538
Pipet, volumetric, 20.00-mL, Class A	each	1451520
Tongs, crucible, 9-inch	each	56900

[1] Other sizes available.

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Edition 7

Carbon Dioxide

DOC316.53.01167

Digital Titrator Method Using Sodium Hydroxide

Method 8205

10 to 1000 mg/L CO_2

Digital Titrator

Scope and Application: For water and seawater.

Test preparation

Before starting the test:

Avoid excess agitation when collecting and swirling the sample to prevent the loss of carbon dioxide from the sample. The sample is measured directly in the Erlenmeyer flask to avoid agitation..
For more accurate results, check the calibration of the Erlenmeyer flask. Fill a graduated cylinder with the sample volume of deionized water. Pour the water into the Erlenmeyer flask and make a mark at the correct level.
Four drops of Phenolphthalein Indicator Solution[1] can be substituted for the Phenolphthalein Indicator Powder Pillow.
A pH meter can be used in place of the indicator. The end point pH is 8.3.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

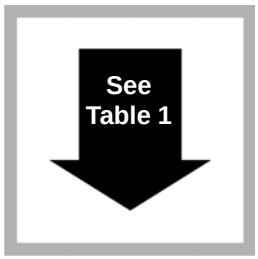
[1] See *Optional reagents and apparatus*.

Collect the following items:

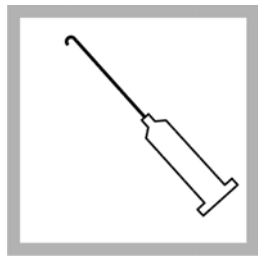
Description	Quantity
Phenolphthalein Indicator Powder Pillow	1 pillow
Sodium hydroxide titration cartridge (see *Range-specific information*)	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Carbon dioxide



1. Select a sample volume and titration cartridge from the *Range-specific information* table.



2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.

4. Collect a water sample directly into the titration flask by filling to the appropriate mark.

Carbon dioxide (continued)

5. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix.

If a pink color forms, no carbon dioxide is present.

6. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from colorless to a light pink color that persists for 30 seconds (pH 8.3).

Write down the number of digits displayed on the counter.

7. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L as CO_2

Example: 100 mL of sample was titrated with the 0.3636 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.2 = 50 mg/L CO_2

Table 62 Range-specific information

Range (mg/L as CO_2)	Sample volume (mL)	Titration cartridge (N NaOH)	Multiplier
10–50	200	0.3636	0.1
20–100	100	0.3636	0.2
100–400	200	3.636	1.0
200–1000	100	3.636	2.0

Interferences

Interfering substances lists substances that can interfere with this test.

Table 63 Interfering substances

Interfering substance	Interference level
Other acids	Other acid components in the sample will be titrated and interfere directly in this test.
Color or turbidity	Color or turbidity can mask the color change of the end point. Use a pH meter instead of the color indicators and titrate to a pH of 8.3.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Fill completely and cap tightly.
- Prevent excessive agitation or prolonged exposure to air.
- Complete the test procedure as soon as possible after collection for best accuracy.
- The sample can be stored for at least 24 hours if cooled to 4 °C (39 °F) or below.
- Warm to room temperature before the test is started.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Carbon Dioxide Voluette® Ampule Standard Solution, 10,000 mg/L CO_2
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL and Pipet Tips

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 0.1 mL of standard that was added will use approximately 50 digits of the 0.3636 N titration cartridge or 5 digits of the 3.636 N titration cartridge to reach the endpoint. If more or less titrant was used, there may be an interference (see *Interferences*) or the concentration of the titrant has changed.

Summary of method

Acidity due to carbon dioxide in a sample is titrated with sodium hydroxide to a phenolphthalein end point. Strong acids are assumed to be absent or of insignificant concentration.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Carbon Dioxide Reagent Set (approximately 100 tests)			2272700
(1) Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
(1) Sodium Hydroxide Titration Cartridge, 0.3636 N	varies	each	1437801
(1) Sodium Hydroxide Titration Cartridge, 3.636 N	varies	each	1438001

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546

Recommended standards

Description	Unit	Catalog number
Carbon Dioxide Standard Solution, Voluette® Ampule, 10,000-mg/L as CO_2, 10-mL	16/pkg	1427510

Optional reagents and apparatus

Description	Unit	Catalog number
Phenolphthalein Indicator Solution, 5-g/L	100 mL MDB	16232
Potassium Acid Phthalate Standard Solution, 100-mg/L as CO_2	100 mL	226142
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Tips for Tensette	50/pkg	2185696
Water, deionized	500 mL	27249
Cylinder, graduated, 250 mL	each	108146
Bottle, sampling, poly, 500 mL	each	2087079
pH meter	each	—
Thermometer, -10 to 220 °C	each	2635700
Ampule Breaker	each	2196800
TitraStir Apparatus, 115 Vac	each	1940000
TitraStir Apparatus, 230 Vac	each	1940010
Digital Titrator delivery tips	5/pkg	1720500

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Edition 7

Carbon Dioxide

DOC316.53.01152

Buret Titration Method[1]

Method 8223

0 to 250 mg/L

Buret Titration

Scope and Application: For water and seawater.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

Avoid excess agitation when collecting and swirling the sample to prevent the loss of carbon dioxide. Measure the sample directly in the Erlynmeyer flask to avoid agitation.
For more accurate results, check the calibration of the Erlenmeyer flask. Fill a graduated cylinder with the sample volume of deionized water. Pour the water into the Erlenmeyer flask and mark the correct level with a wax pencil or permanent marker.
Four drops of Phenolphthalein Indicator Solution[1] can be substituted for the Phenolphthalein Indicator Powder Pillow.
The concentration of the sodium hydroxide standard solution will slowly decrease. To prevent deterioration, keep the bottle tightly sealed after use. Fill the buret immediately before the test is started and discard the remaining solution after the test. Follow the *Accuracy check* each month to make sure the solution will give accurate results.
A pH meter can be used in place of the indicators. The end point pH is 8.3.
For added convenience when stirring, use the TitraStir® stirring apparatus.

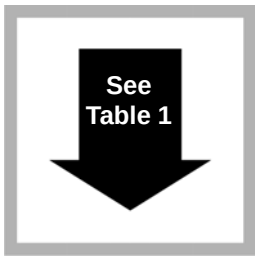
[1] See *Optional reagents and apparatus*.

Collect the following items:

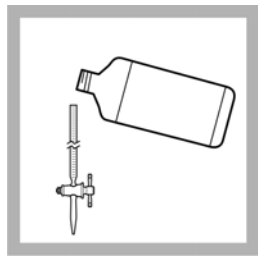
Description	Quantity
Phenolphthalein Indicator Powder Pillow	1
Sodium Hydroxide Standard Solution, 0.0227 N	1 bottle
Buret, Class A, 25-mL, with support stand	1
Erlenmeyer flask	1

See *Consumables and replacement items* for reorder information.

Buret titration



1. Select a sample volume and flask from the *Range-specific information* table.



2. Fill a 25-mL buret to the zero mark with 0.0227 N Sodium Hydroxide Standard Solution.

3. Fill a clean Erlenmeyer flask to the selected volume. If collected from a faucet, allow the sample to overflow several times and then pour off the excess sample.

4. Add the contents of one Phenolphthalein Indicator Powder Pillow and mix gently.

5. Titrate the sample while gently swirling the flask until a light pink color forms and persists for 30 seconds.

6. Calculate:

mL titrant used x multiplier = mg/L as CO_2

Example: 100 mL of sample was titrated and 15 mL of titrant was used to reach the endpoint. The concentration is 15 x 10 = 150 mg/L as CO_2

Table 64 Range-specific information

Range (mg/L as CO_2)	Sample volume (mL)	Flask size	Multiplier
0–125	200	250	5
100–250	100	125	10

Interferences

Interfering substances lists substances that can interfere with this test.

Table 65 Interfering substances

Interfering substance	Interference level
Color or turbidity	Color or turbidity can mask the color change of the end point. Use a pH meter instead of the phenolphthalein indicator and titrate to a pH of 8.3.
Acids	Acids other than carbonic acid will be titrated and interfere directly.

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation or prolonged exposure to air. Complete the test procedure as soon as possible after collection for best accuracy. The sample can be stored for at least 24 hours if cooled.

Accuracy check

The standard additions method can be used to find if the sample has an interference. The standard solution method can be used to confirm analytical technique and reagent performance.

Standard additions method (sample spike)
Required for accuracy check:

- Carbon Dioxide Voluette® Ampule Standard Solution, 10,000-mg/L as CO_2
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL and Pipet Tips.

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 0.1 mL of standard that was added will use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there may be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Standard solution method
A sodium hydroxide standard solution will slowly absorb carbon dioxide from the atmosphere, which forms carbonic acid and neutralizes some of the hydroxide. When sealed in plastic bottles, the sodium hydroxide concentration will decrease by approximately 1% per year. Complete the following test to make sure the concentration is accurate.

Required for accuracy check:

- Potassium Acid Phthalate Standard Solution, 100-mg/L as CO_2

1. Add 50.0-mL of Potassium Acid Phthalate Standard Solution, 100-mg/L as CO_2 to an Erlenmeyer flask.
2. Add the phenolphthalein indicator and swirl to mix.
3. Titrate the standard to the end point with the sodium hydroxide standard solution. The titration should require 5.00 mL of sodium hydroxide solution. If the volume is greater than 5.25 mL, discard the sodium hydroxide and replace it with a fresh supply.

   Note: *To correct for a reduction in strength, divide 5.00 by the number of mL required for this titration. Multiply all sample results by this factor.*

Summary of method

The analysis for dissolved carbon dioxide in water is similar to that for acidity. A water sample is titrated to a phenolphthalein end point at pH 8.3 with a sodium hydroxide standard solution. Strong mineral acids are assumed to be absent or their effect to be negligible.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
Sodium Hydroxide Standard Solution, 0.0227 N	varies	1 L	19253

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Support Stand	1	each	56300

Recommended standards

Description	Unit	Catalog number
Carbon Dioxide Standard Solution, Voluette® Ampule, 10,000-mg/L as CO_2, 10-mL	16/pkg	1427510
Potassium Acid Phthalate Standard Solution, 100-mg/L as CO_2	100 mL	226142

Optional reagents and apparatus

Description	Unit	Catalog number
Phenolphthalein Indicator Solution, 5-g/L	100 mL	16232
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Water, deionized	500 mL	27249
Cylinder, graduated, 250 mL	each	108146
Sampling Bottle with cap, low density polyethylene, 500 mL	12/pkg	2087079
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Voluette Ampule breaker 10 mL	each	2196800
TitraStir Apparatus, 115 Vac	each	1940000
TitraStir Apparatus, 230 Vac	each	1940010
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352

[1] Other sizes are available

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Edition 7

Chelant, Free

DOC316.53.01168

Digital Titrator Using Magnesium Chloride

Method 8352

0 to 20.0 mg/L as $CaCO_3$

Digital Titrator

Scope and Application: For industrial waters.

Test preparation

Before starting the test:

All apparatus must be extremely clean and rinsed frequently with acid and deionized water to remove any hardness present on the plastic or glass.
Filter turbid samples with filter paper[1] and a funnel.
Four drops of ManVer Hardness Indicator Solution[1] or a 0.1 g scoop of ManVer 2 Hardness Indicator Powder[1] can be used in place of the ManVer 2 Hardness Indicator Powder Pillow.
For best results, measure a reagent blank. Use 50 mL of deionized water in place of the sample. Subtract the number of digits that were used for the reagent blank from the number of digits that were used for titrating the sample.
Results may be expressed as mg/L tetrasodium EDTA. (Digits required x 0.38 = mg/L as Na_4 EDTA)
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

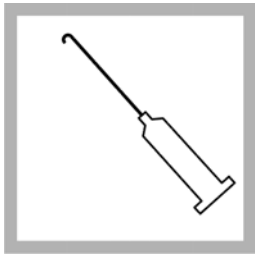
[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
Hardness 1 Buffer Solution	1 bottle
ManVer 2 Hardness Indicator Powder Pillow	1 pillow
Magnesium Chloride Titration Cartridge, 0.0800 M	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 125-mL	1

See *Consumables and replacement items* for reorder information.

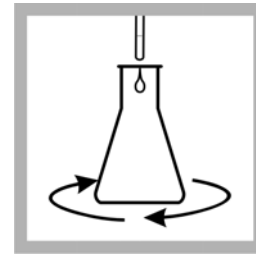
Free chelant



7. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

8. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

9. Use a graduated cylinder to measure 100 mL of sample into a 125-mL Erlenmeyer flask.



10. Add two full droppers (2 mL) of Hardness 1 Buffer Solution to the flask. Swirl to mix.

11. Add the contents of one ManVer 2 Hardness Indicator Powder Pillow. Swirl to mix.

If the solution turns blue, free chelant is present. If the solution turns red, a chelant deficiency exists.

12. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes to red-violet.

Write down the number of digits on the counter.

13. Calculate:

digits x 0.1 = mg/L Free Chelant as $CaCO_3$

Example: 100 mL of sample was titrated with the 0.0800 M cartridge and 120 digits were used to reach the endpoint. The concentration is 120 x 0.1 = 12 mg/L as $CaCO_3$

Interferences

Interfering substances lists substances that can interfere with this test.

Table 66 Interfering substances

Interfering substance	Interference level
Orthophosphate	Causes a slow end point.
Polyphosphate	Must be absent for accurate results.
pH	If chelant residual in boiler water is being analyzed, adjust the pH before adding the Buffer Solution, Hardness 1 as follows: **1.** Measure 100 mL of sample into a flask. **2.** Add 2 drops of Phenolphthalein Indicator Solution. **3.** Add 5.25 N Sulfuric Acid Standard Solution by drops until the solution changes from pink to colorless. Write down the number of drops that were added. Discard this sample. **4.** To a fresh 100-mL portion of sample, add the same number of drops of 5.25 N Sulfuric Acid Standard Solution before adding the buffer in step 10 of the test.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Filter the sample if turbid with filter paper and a funnel.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- EDTA Standard Solution, 0.035 N
- TenSette Pipet, 0.1–1.0 mL

4. Use the TenSette Pipet to add 0.4 mL of the standard to the titrated sample. Swirl to mix.

5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

6. Each 0.4 mL of standard that was added will use approximately 70 digits of the titration cartridge to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed.

Summary of method

Chelant residual is determined by titration with a standard solution of magnesium chloride at pH 10. The end point is determined by a color change from blue to red-violet.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hardness 1 Buffer Solution	2 mL	100 mL	42432
ManVer® 2 Hardness Indicator Powder Pillows	1 pillow	100/pkg	85199
Magnesium Chloride Titration Cartridge, 0.0800 M	varies	each	2062501

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Cylinder, graduated, 100-mL	1	each	50842

Recommended standards

Description	Unit	Catalog number
EDTA Standard Solution, 0.035 N	100 mL	2349932

Optional reagents and apparatus

Description	Unit	Catalog number
Delivery tubes, with 180° hook	5/pkg	1720500
Delivery tubes, with 90° hook	5/pkg	4157800
Filter paper, folded, 12.5 cm	100/pkg	189457
Funnel, analytical, poly, 65 mm	each	108367
ManVer 2 Hardness Indicator Powder	113 g	28014
ManVer Hardness Indicator Solution	100 mL	42532
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Phenolphthalein indicator solution, 5 g/L	15 mL SCDB	16236
Sulfuric acid standard solutions, 5.25 N	100 mL MDB	244932

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Edition 7

Chelant, Total

DOC316.53.01169

Bismuth Nitrate Method **Method 8350**

0 to 40.0 mg/L as Na_4 EDTA **Digital Titrator**

Scope and Application: For industrial waters.

Test preparation

Before starting the test:

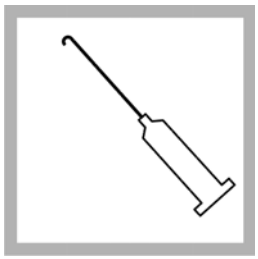
Filter turbid samples with filter paper and a funnel.
For best results, measure a reagent blank. Use 50 mL of deionized water in place of the sample. Subtract the number of digits that were used for the reagent blank from the number of digits that were used for titrating the sample.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

Collect the following items:

Description	Quantity
Methylthymol Blue Indicator Powder Pillow	1 pillow
Ascorbic Acid Powder Pillow	1 pillow
Bismuth Nitrate Titration Cartridge, 0.0200 M	1 cartridge
Sulfuric Acid Standard Solution, 5.25 N	1 bottle
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder, 100-mL	1
Erlenmeyer flask, 125-mL	1

See *Consumables and replacement items* for reorder information.

Total chelant

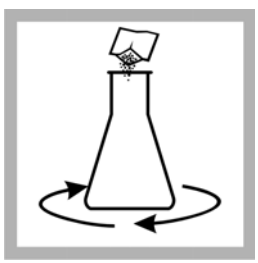


14. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

15. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

16. Use a graduated cylinder to measure 50 mL of sample into a 125-mL Erlenmeyer flask.

17. Add the contents of one Ascorbic Acid Indicator Powder Pillow. Swirl to mix.



18. Add the contents of one Methylthymol Blue Indicator Powder Pillow. Swirl to mix.

19. If the solution in the flask is yellow, add one drop of 5.25 N Sulfuric Acid Standard Solution.

If the solution is blue, add 5.25 N Sulfuric Acid Standard solution by drops until the color changes to yellow. Add one additional drop.

20. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from yellow to blue-green. Titrate slowly as the end point is approached.

Write down the number of digits displayed on the counter.

21. Calculate:

digits x 0.188 = mg/L total chelant as Na_4 EDTA

Example: 50 mL of sample was titrated and 120 digits were used to reach the endpoint. The concentration is 120 x 0.188 = 22.3 mg/L total chelant as Na_4 EDTA

Interferences

Interference from ferric iron (Fe^{3+}) is minimized by the addition of ascorbic acid. Approach the end point slowly in samples that contain ferric iron because the reduced iron decreases the sharpness of the color change.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Filter the sample if turbid with filter paper and a funnel.

Summary of method

Total chelant is determined by titrating an acidic sample with bismuth nitrate in the presence of methylthymol blue indicator. The end point is indicated by a color change from yellow to blue-green.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ascorbic Acid Powder Pillows	1 pillow	100/pkg	1457799
Bismuth Nitrate Titration Cartridge, 0.0200 M	varies	each	2434501
Methylthymol Blue Indicator Powder Pillows	1 pillow	100/pkg	2284799
Sulfuric Acid Standard Solution, 5.25 N	varies	100 mL MDB	244932

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Cylinder, graduated, poly, 100-mL	1	each	108142
Delivery tubes, with 180° hook	1	5/pkg	1720500
Delivery tubes, with 90° hook	1	5/pkg	4157800

Optional reagents and apparatus

Description	Unit	Catalog number
Filter paper, folded, 12.5 cm	100/pkg	189457
Funnel, analytical, poly, 65 mm	each	108367
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Sampling bottle	250 mL	2087076

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Edition 7

Chloramine (Mono); Nitrogen, Free Ammonia

DOC316.53.01016

Indophenol Method[1] **Method 10200**

(0.01–0.50 mg/L NH_3–N; 0.04–4.50 mg/L Cl_2) **Powder Pillows**

Scope and Application: For determining Free Ammonia and Monochloramine simultaneously in finished chloraminated water.

[1] U.S. Patent 6,315,950

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 67 Instrument-specific information

Instrument	Sample cell	Cell orientation	Adapter
DR 6000	4864302	Orientation key toward user	A23618
DR 5000	4864302	Orientation key toward user	A23618
DR 3900	4864302	Orientation key inserted in adapter slot	LZV846 (A)
DR 3800, DR 2800, DR 2700	5940506	1-cm (flat) path aligned with the arrow on the adapter	LZV585 (B)

Before starting the test:

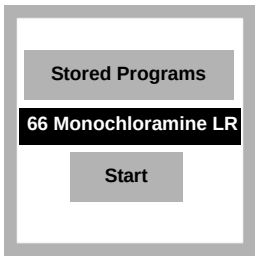
For more accurate chloramine results, determine a reagent blank value for each new lot of reagent, using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.
Dispose of reacted solutions according to local, state and federal regulation. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.

Collect the following items:

Description	Quantity
Free Ammonia Reagent Solution	1 drop
Monochlor F Reagent Pillows	2
Sample cell (see *Instrument-specific information*)	2

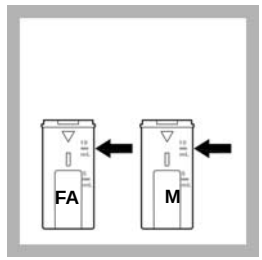
See *Consumables and replacement items* for reorder information.

Indophenol method, powder pillows



1. Select the Monochloramine test.

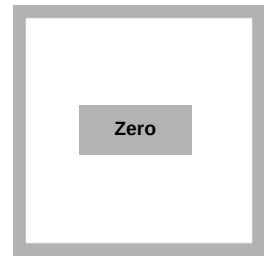
Insert an adapter if required (see *Instrument-specific information*).



2. Fill two 1-cm cells to the 10-mL line with sample.

Label one cell “Free Ammonia” and one cell “Monochloramine”.

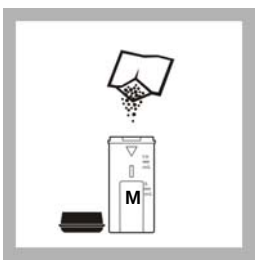
3. Wipe the monochloramine cell and insert it into the cell holder. See *Instrument-specific information* for cell orientation



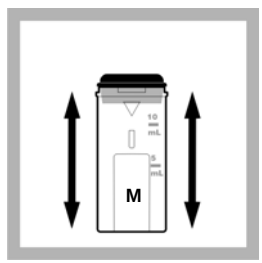
4. **ZERO** the instrument.

The display will show:

0.00 mg/L Cl_2

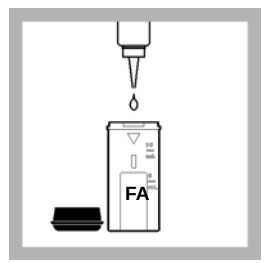


5. Remove the cell and add the contents of one Monochlor-F pillow to the sample for monochloramine measurement.



6. Cap and shake the cell about 20 seconds to dissolve.

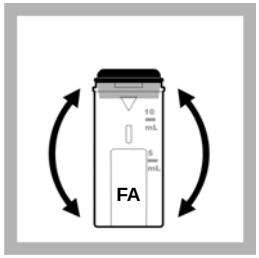
A green color will develop if monochloramine is present.



7. Add one drop of Free Ammonia Reagent Solution to the cell for Free Ammonia measurement.

8. Cap the reagent bottle to maintain reagent performance and stability.

Indophenol method, powder pillows (continued)



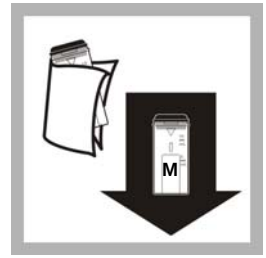
9. Cap and invert the Free Ammonia cell to mix.

If the sample becomes cloudy by the end of the reaction period, pretreat the sample and retest. See the *Interferences* section.

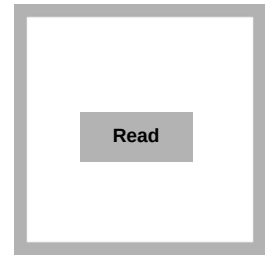
10. Start the instrument timer.

A 5-minute reaction period will begin.

Color development time depends on sample temperature. For accurate results allow the full reaction period to occur. See the *Color development* table

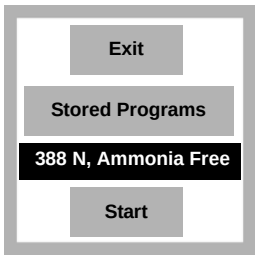


11. When the timer expires, wipe the **Monochloramine** cell and insert it into the cell holder.



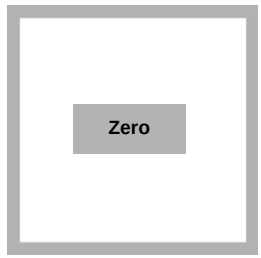
12. READ the results in mg/L Monochloramine (as Cl_2).

Leave the cell in the instrument.



13. Select the Free Ammonia test.

If Display Lock is on, the display will show “Store Data?”. Press **YES** or **NO** and continue.



14. With the monochloramine sample still in the cell holder, **ZERO** the instrument.

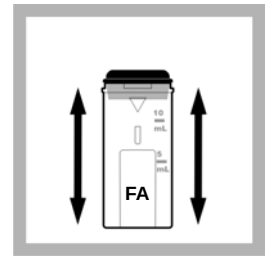
The display will show:

0.00 mg/L NH_3–N f

Remove the monochloramine cell.

15. Add the contents of one Monochlor F pillow to the cell for the Free Ammonia measurement.

The reaction period in step 10 must be complete before adding the Monochlor F to the cell for Free Ammonia measurement.



16. Cap and shake the cell about 20 seconds to dissolve the reagent.

A green color will form if monochloramine or ammonia is present.

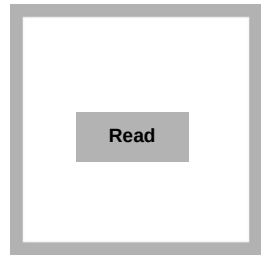
Indophenol method, powder pillows (continued)

17. Start the instrument timer.

A 5-minute reaction period will begin.

Samples colder than 18 °C will require additional time. See the *Color development* table.

18. When the timer expires, insert the vial into the cell.



19. **READ** the results in mg/L NH_3–N f.

Interferences

This method is intended for finished, chloraminated drinking water samples that have a measurable combined (total) chlorine disinfectant residual. Samples where the disinfectant residual has disappeared and samples which exhibit a chlorine demand may produce low ammonia test results. Blanks and ammonia standards analyzed without a disinfectant residual must be prepared using high quality, reagent grade water.

The substances listed in the *Non-interfering substances* table do not interfere in free ammonia determination at or below the stated concentration:

Table 68 Non-interfering substances

Substance	Maximum level tested
Aluminum	0.2 mg/L Al
Chloride	1200 mg/L Cl^-
Copper	1 mg/L Cu
Iron	0.3 mg/L Fe
Manganese	0.05 mg/L Mn
Nitrate	10 mg/L NO_3^-–N
Nitrite	1 mg/L NO_2^-–N
Phosphate	2 mg/L o–PO_4
Silica	100 mg/L SiO_2
Sulfate	1600 ppm as SO_4^{2-}
Zinc	5 ppm Zn

Samples containing high levels of both Total Hardness and Alkalinity may become cloudy after the addition of the Free Ammonia Reagent Solution. If this occurs by the end of the first reaction period, the sample for Free Ammonia measurement must be pretreated as follows:

Note: *The sample for Monochloramine measurement does not need pretreatment.*

1. Measure 10 mL of sample into the cell for Free Ammonia measurement.
2. Add the contents of one Hardness Treatment Reagent Powder Pillow to the sample.
3. Cap the cell and invert until the reagent is dissolved.
4. Remove the cap.
5. Continue with the analysis at step 2 using the pretreated sample as the Free Ammonia cell.

Color development time

Test results are strongly influenced by sample temperature. **Both reaction periods in the procedure are the same and depend on the temperature of the sample.** The reaction periods indicated in the procedure are for a sample temperature of 18–20 °C (64–68 °F). Adjust both reaction periods according to the *Color development* table. Samples can be read up to 15 minutes after the listed development times.

Table 69 Color development

Sample temperature (°C)	Sample temperature (°F)	Development time (minutes)
5	41	10
7	45	9
9	47	8
10	50	8
12	54	7
14	57	7
16	61	6
18	64	5
20	68	5
23	73	2.5
25	77	2
greater than 25	greater than 77	2

Sampling and storage

Collect samples in clean glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.

Accuracy check (Monochloramine, Program 66)

Required for accuracy check:

- Buffer Powder Pillow, pH 8.3
- Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N
- Chlorine Solution Ampules, 50–70 mg/L
- 100-mL Class A volumetric flask
- Pipet, TenSette®, 0.1–1.0 mL and tips

- Pipet, Volumetric 2 mL and pipet bulb
- Organic-free water

Standard solution method

Important Note: *Because of the strong buffer used in the preparation of this standard, it cannot be used for accuracy verification of the Free Ammonia test.*

To check test accuracy, prepare the following 4.5-mg/L (as Cl_2) monochloramine standard immediately before use.

1. Add the contents of one Buffer Powder Pillow, pH 8.3 to about 50-mL of organic-free water in a clean 100-mL Class A volumetric flask. Swirl to dissolve the powder.
2. Using a Class A volumetric pipet, transfer 2.00 mL of Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N into the flask.
3. Dilute to volume with organic-free water, cap and mix thoroughly. This is a 2.00-mg/L buffered ammonia standard.
4. Pipet 50.00 mL of the buffered ammonia standard into a clean 100-mL beaker. Add a stir bar.
5. Obtain a recent lot of Chlorine Solution Ampules, 50–70 mg/L, and note the actual free chlorine concentration for this lot.
6. Calculate the amount of Chlorine Solution to be added to the ammonia standard using the following equation:

$$\text{mL chlorine solution required} = \frac{455}{\text{free chlorine concentration}}$$

7. Open an ampule and use a glass Mohr pipet to add the calculated amount of Chlorine Solution slowly to the ammonia standard, while mixing at medium speed on a stir-plate.
8. Allow the monochloramine solution to mix for 1 minute after all Chlorine Solution is added.
9. Quantitatively transfer the monochloramine solution to a clean 100-mL Class A volumetric flask. Dilute to the mark with organic-free water, cap, and mix thoroughly. This is a nominal 4.5-mg/L (as Cl_2) monochloramine standard.
10. Use this standard within 1 hour of preparation. Analyze according to the Low Range Monochloramine procedure described above.
11. To adjust the calibration curve using the reading obtained with the 4.5-mg/L standard solution, select Options>More>Standard Adjust from the instrument menu.
12. Turn on the Standard Adjust feature and accept the shown concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Accuracy check (Free Ammonia, Program 388)

Required for accuracy check:

- Ammonium Nitrogen Standard, 10 mg/L as NH_3-N
- 100-mL plastic volumetric flask with stopper
- 50-mL mixing cylinders, three
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Deionized water

Dilution water is required when testing a diluted sample and preparing standard solutions. Dilution water must be free of ammonia, chlorine and chlorine demand. A convenient source is a recirculating, deionizer system with carbon filtration which produces 18 megaohm-cm water.

Standard Additions Method

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Select Options>More>Standard additions from the instrument menu.
3. Default values for standard concentration, sample volume, and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare three spiked samples. Measure 50 mL of sample into three 50-mL mixing cylinders.
5. Use the TenSette Pipet to add 0.3, 0.6, and 1.0 mL of Ammonium Nitrogen Standard, 10 mg/L as NH_3-N to the three samples. Mix well.
6. Analyze each spiked sample starting with the 0.3 mL sample spike. Follow all steps in Method 10200. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solution Method

1. Prepare a 0.20 mg/L ammonia nitrogen standard by diluting 2.00 mL of the Ammonia Nitrogen Standard Solution, 10 mg/L, to 100 mL with dilution water. Or, using the TenSette Pipet, prepare a 0.20 mg/L ammonia nitrogen standard by diluting 0.4 mL of a Ammonia Nitrogen Voluette Standard Solution, 50 mg/L as NH_3–N, to 100 mL with dilution water. Analyze the Standard Solution, following all steps in Method 10200.
2. To adjust the calibration curve using the reading obtained with the standard solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
66	2.60 mg/L Cl_2	2.58–2.62 mg/L Cl_2	0.04 mg/L Cl_2
388	0.20 mg/L NH_3–N	0.19–0.21 mg/L NH_3–N	0.01 mg/L NH_3–N

Summary of method

Monochloramine (NH_2Cl) and free ammonia (NH_3 and NH_4^+) can exist in the same water sample. Added hypochlorite combines with free ammonia to form more monochloramine. In the presence of a cyanoferrate catalyst, monochloramine in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample. Free ammonia is determined by comparing the color intensities, with and without added hypochlorite. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Free Ammonia Reagent Set, includes:	—	50/pkg	2879700
(1) 2802299, (1) 2877336			
Free Ammonia Reagent Solution	1 drop	4 mL SCDB	2877336
Monochlor F Reagent Pillows	2	100/pkg	2802299

Recommended standards and reagents

Description	Unit	Catalog number
Buffer, pH 8.3 Powder Pillows	25/pkg	89868
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
Hardness Treatment Reagent Pillows (1 per test)	50/pkg	2882346
Nitrogen Ammonia Standard Solution, 10 mg/L as NH_3–N	500 mL	15349
Nitrogen Ammonia Standard Ampule, 50 mg/L as NH_3–N, 10 mL	16/pkg	1479110
Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N	500 mL	2406549
PourRite Ampule Breaker, for 2-mL ampules	each	248460
Voluette Ampule Breaker, for 10-mL ampules	each	2196800
Water, Organic-free	500-mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
Beaker, 100 mL, Polypropylene	each	108042
Beaker, 100 mL, Glass	each	50042H
Cylinder, 50 mL, mixing	each	2088641
Flask, volumetric, Class A, 100 mL	each	1457442
Free Ammonia Reagent Set	250/pkg	2879701
Monochloramine/Free Ammonia Spec Check Kit	each	2507500
Pipet Filler, safety bulb	each	1465100
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, Mohr, Glass, 10 mL	each	2093438
Pipet, volumetric, Class A, 2 mL	each	1451536
Pipet, volumetric, Class A, 50 mL	each	1451541
Scissors	each	2883100
Stir Bar, octagonal	each	2095352
Stirrer, magnetic	each	2881200
Thermometer, –10 to 110 °C	each	187701
Wipers, Disposable, 30 x 30 cm, 280/box	box	2097000

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Edition 7

Chloramine (Mono)

DOC316.53.01014

Indophenol Method[1] **Method 10172**

HR (0.1 to 10.0 mg/L Cl_2) **Test 'N Tube**

Scope and Application: Chlorinated wastewater.

[1] Patent Pending

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 70 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield into compartment #2 before performing this test. See the user manual for more information.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Dispose of reacted solutions according to local, state and federal regulations. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.

Collect the following items:

Description	Quantity
High-range Monochloramine Diluent Vials	1
Monochlor F Reagent Pillow	1
Funnel, micro	1
Light Shield (see *Instrument-specific information*)	1
Pipet, Mohr glass, 2.00 mL and pipet bulb	1
Test Tube Rack	1

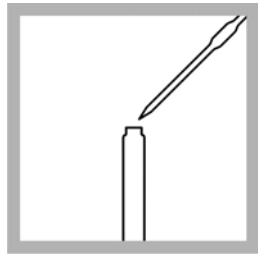
See *Consumables and replacement items* for reorder information.

Indophenol method for monochloramine

20. Select the test.

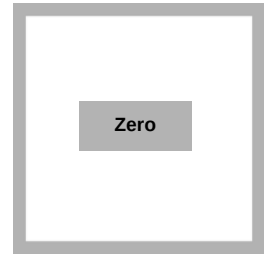
Insert an adapter or light shield if required (*Instrument-specific information*).

Refer to the user manual for orientation.



21. Remove the cap from one HR Monochloramine Diluent vial. Use a glass pipet to add 2.0 mL sample to the vial. Re-cap and invert several times to mix.

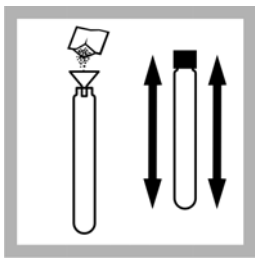
22. Wipe the vial and insert it into the 16 mm cell holder.



23. **ZERO** the instrument.

The display will show:

0.0 mg/L Cl_2

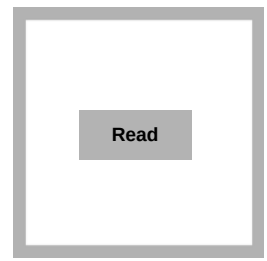


24. Remove the vial from the holder. Using a micro-funnel, add the contents of one Monochlor F pillow to the sample. Cap and shake the cell about 20 seconds to dissolve.

25. Start the instrument timer.

A 5-minute reaction period will begin.

26. After the timer expires, re-insert the vial into the cell holder.



27. **READ** the results in mg/L Cl_2.

Interferences

The substances in the *Non-interfering substances* table have been tested for interference and do not interfere at or below the indicated levels. The *Interfering substances* table describes suggested treatments for interfering substances.

Table 71 Interfering substances

Interfering substance and effects		Interference level	Recommended treatment
Ozone	–	Above 1 mg/L	Usually does not coexist with monochloramine.
Sulfide	+	Turns a “rust” color if present.	Usually does not coexist with monochloramine.
Thiocyanate	–	Above 0.5 mg/L	This method will tolerate up to 2 mg/L.

Table 72 Non-interfering substances

Substance	Maximum level tested
Alanine	1 mg/L N
Aluminum	10 mg/L
Bromide	100 mg/L Br^-
Bromine	15 mg/L Br_2
Calcium	1000 mg/L as $CaCO_3$
Chloride	18,000 mg/L Cl^-
Chlorine Dioxide	5 mg/L ClO_2
Chromium (III)	5 mg/L Cr
Copper	10 mg/L Cu
Cyanide	10 mg/L CN^-
Dichloramine	10 mg/L as Cl_2
Fluoride	5 mg/L F^-
Free Chlorine	10 mg/L Cl_2
Glycine	1 mg/L N
Iron (II)	10 mg/L Fe^{2+}
Iron (III)	10 mg/L Fe^{3+}
Magnesium	1000 mg/L as $CaCO_3$
Manganese (VII)	10 mg/L
Lead	10 mg/L Pb
Nitrate	100 mg/L N
Nitrite	50 mg/L N
Phosphate	100 mg/L PO_4
Silica	100 mg/L SiO_2
Sulfate	2600 mg/L SO_4^{2-}
Sulfite	50 mg/L SO_3^{2-}
Tyrosine	1 mg/L N
Urea	10 mg/L N
Zinc	5 mg/L Zn

Sampling and storage

Analyze samples for monochloramine immediately after collection. Rinse the sample container several times with sample, letting the container overflow each time. If sampling from a tap, let the water flow for at least 5 minutes, then cap the container so that there is no head space (air) above the sample.

Accuracy check

Required for accuracy check:

- Buffer Powder Pillow, pH 8.3
- Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N
- Chlorine Solution Ampules, 50–70 mg/L Cl_2
- 100-mL Class A volumetric flask
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Pipet, Volumetric, 2 mL and pipet bulb
- Organic-free water

To check test accuracy, prepare the following 4.5-mg/L (as Cl_2) monochloramine standard immediately before use.

1. Add the contents of one Buffer Powder Pillow, pH 8.3 to about 50-mL of organic-free water in a clean 100-mL Class A volumetric flask. Swirl to dissolve the powder.
2. Using a Class A volumetric pipet, transfer 2.00 mL of Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N into the flask.
3. Dilute to volume with organic-free water, cap and mix thoroughly. This is a 2.00-mg/L buffered ammonia standard.
4. Pipet 50.00 mL of the buffered ammonia standard into a clean 100-mL beaker. Add a stir bar.
5. Obtain a recent lot of Chlorine Solution Ampules, 50–75 mg/L, and note the actual free chlorine concentration for this lot.
6. Calculate the amount of Chlorine Solution to be added to the ammonia standard using the following equation:

$$\text{mL chlorine solution required} = \frac{455}{\text{free chlorine concentration}}$$

7. Open an ampule and use a glass Mohr pipet to add the calculated amount of Chlorine Solution slowly to the ammonia standard, while mixing at medium speed on a stir plate.
8. Allow the monochloramine solution to mix for 1 minute after all Chlorine Solution is added.
9. Quantitatively transfer the monochloramine solution to a clean 100-mL Class A volumetric flask. Dilute to the mark with organic-free water, cap, and mix thoroughly. This is a nominal 4.5-mg/L (as Cl_2) monochloramine standard.
10. Use this standard within 1 hour of preparation. Follow the *Indophenol method for monochloramine* test.
11. To adjust the calibration curve using the reading obtained with the 4.5-mg/L standard solution, select Options>More>Standard Adjust from the instrument menu.
12. Turn on the Standard Adjust feature and accept the shown concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
67	5.9 mg/L Cl_2	5.6–6.2 mg/L Cl_2	0.1 mg/L Cl_2[1]

[1] Use the LR Chloramine (Mono) test for concentrations below 4.5 mg/L Cl_2.

Summary of Method

The sample is first diluted in a Test 'N Tube. In the presence of a cyanoferrate catalyst, monochloramine (NH_2Cl) in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
HR Monochloramine Test 'N Tubes, 50 tests, includes:	—	—	2805145
(50) HR Monochloramine Diluent Vials[1]	1	50/pkg	—
Funnel, micro	1	each	2584335
Monochlor F Reagent Pillows	1	50/pkg	2802246

[1] Not sold separately

Required apparatus

Description	Quantity	Unit	Catalog number
Pipet, Mohr, glass 2.00 mL	1	each	2093636
Piper Filler, safety bulb	—	each	1465100
Test Tube Rack	1–2	each	1864100

Recommended standards and apparatus

Description	Unit	Catalog number
Buffer Powder Pillows, pH 8.3	25/pkg	89868
Chlorine Solution Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
Chlorine Solution 2 mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Chlorine Solution 2 mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N	500 mL	2406549
PourRite Ampule Breaker, for 2-mL ampules	each	2484600
Voluette Ampule Breaker, for 10-mL ampules	each	2196800
Water, organic-free	500 mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
Beaker, 100 mL	each	50042H
Clippers	each	96800
Flask, volumetric, Class A, 100-mL	each	1457442
Pipet, Mohr, glass, 5-mL	each	2093437
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips for TenSette Pipet	50/pkg	2185696
Pipet Tips for TenSette Pipet	1000/pkg	2185628
Pipet, volumetric, Class A, 2-mL	each	1451536
Pipet, volumetric, Class A, 50-mL	each	1451541
Stir bar, octagonal	each	2095352
Stirrer, magnetic	each	2881200
Shears	each	2369400

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Edition 7

Chloramine (Mono)

DOC316.53.01015

Indophenol Method[1] **Method 10171**

LR (0.04 to 4.50 mg/L Cl_2) **Powder Pillows**

Scope and Application: Chloraminated drinking water and chlorinated wastewater

[1] U.S. Patent 6,315,950

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 73 Instrument-specific information

Instrument	Sample cell	Cell orientation	Adapter
DR 6000	4864302	Orientation key toward user	A23618
DR 5000	4864302	Orientation key toward user	A23618
DR 3900	4864302	Orientation key away from user	LZV846 (A)
DR 3800, DR 2800, DR 2700	5940506	1-cm (flat) path aligned with the arrow on the adapter	LZV585 (B)

Before starting the test:

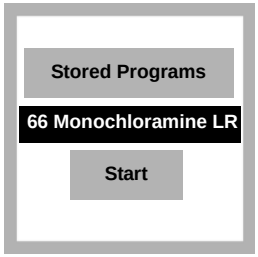
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
To determine chloramine (mono) and free ammonia on the same sample, use Method 10200 Nitrogen, Free Ammonia and Chloramine (Mono)
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.
Dispose of reacted solutions according to local, state and federal regulation. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.

Collect the following items:

Description	Quantity
Monochlor F Reagent Pillow	1
Sample Cell (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

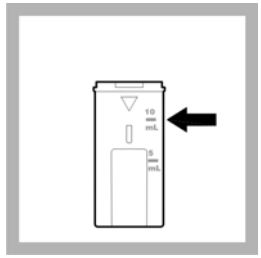
Indophenol method, powder pillows



1. Select the test.

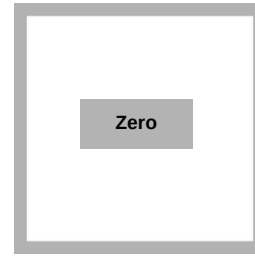
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill the cell to the 10-mL line with sample.

3. Wipe the cell and insert it into the cell holder.

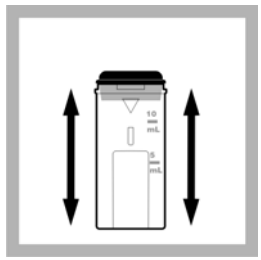


4. **ZERO** the instrument.

The display will show:

0.00 mg/L Cl_2

5. Remove the cell and add the contents of one Monochlor-F pillow to the sample.



6. Cap and shake the cell about 20 seconds to dissolve.

7. Start the instrument timer.

A 5-minute reaction period will begin. Samples colder than 18 °C will require additional time. See the *Color development time* table.

8. After the timer expires, insert the vial into the cell.

READ the results in mg/L Cl_2.

Interferences

The substances listed in the *Non-interfering substances* table have been tested for interference and do not interfere at or below the indicated levels. The *Interfering substances* table suggests treatments for interferences.

Table 74 Non-interfering substances

Interfering substance	Interference level
Alanine	1 mg/L N
Aluminum	10 mg/L Al
Bromide	100 mg/L Br^-
Bromine	15 mg/L Br_2
Calcium	1000 mg/L as $CaCO_3$
Chloride	18,000 mg/L Cl^-
Chlorine Dioxide	5 mg/L ClO_2
Chromium (III)	5 mg/L Cr^{3+}
Copper	10 mg/L Cu
Cyanide	10 mg/L CN^-
Dichloramine	10 mg/L as Cl_2
Fluoride	5 mg/L F^-
Free Chlorine	10 mg/L Cl_2
Glycine	1 mg/L N
Iron (II)	10 mg/L Fe^{2+}
Iron (III)	10 mg/L Fe^{3+}
Lead	10 mg/L Pb
Nitrate	100 mg/L N
Nitrite	50 mg/L N
Phosphate	100 mg/L PO_4
Silica	100 mg/L SiO_2
Sulfate	2600 mg/L SO_4^{2+}
Sulfite	50 mg/L SO_3^{2-}
Tyrosine	1 mg/L N
Urea	10 mg/L N
Zinc	5 mg/L Zn

Table 75 Interfering substances

Interfering substance and effects		Interference level	Recommended treatment
Magnesium	+	Above 400 mg/L $CaCO_3$	Add 5 drops Rochelle Salt Solution prior to testing. OR: use the high range (HR) test.
Manganese (+7)	–	Above 3 mg/L	Use the HR test; it will tolerate up to 10 mg/L.
Ozone	–	Above 1 mg/L	Usually does not coexist with monochloramine.
Sulfide	+	Turns a “rust” color if present.	Usually does not coexist with monochloramine.
Thiocyanate	–	Above 0.5 mg/L	This method will tolerate up to 2 mg/L.

Color development time

Test results are strongly influenced by sample temperature. The reaction period indicated in the procedure is for a sample temperature of 18–20 ºC (64–68 ºF). Adjust the reaction period according to Table 76. Samples can be read up to 15 minutes after the listed development time.

Table 76 Color development time

Sample temperature		Development time (minutes)
°C	°F	
5	40	10
7	42	9
9	48	8
10	50	8
12	54	7
14	58	7
16	61	6
18	68	4
20	73	3
23	75	2.5
25	77	2
greater than 25	greater than 77	2

Sampling and storage

Analyze samples for monochloramine immediately after collection. Rinse the sample container several times with sample, letting the container overflow each time. If sampling from a tap, let the water flow for at least 5 minutes, then cap the container so that there is no head space (air) above the sample.

Accuracy check

Required for accuracy check:

- Buffer Powder Pillow, pH 8.3
- Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N
- Chlorine Solution Ampules, 50–70 mg/L
- 100-mL Class A volumetric flask
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Pipet, Volumetric, 2 mL and pipet bulb
- Organic-free water

To check test accuracy, prepare the following 4.5-mg/L (as Cl_2) monochloramine standard immediately before use.

1. Add the contents of one Buffer Powder Pillow, pH 8.3 to about 50-mL of organic-free water in a clean 100-mL Class A volumetric flask. Swirl to dissolve the powder.
2. Using a Class A volumetric pipet, transfer 2.00 mL of Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N into the flask.

3. Dilute to volume with organic-free water, cap and mix thoroughly. This is a 2.00-mg/L buffered ammonia standard.
4. Pipet 50.00 mL of the buffered ammonia standard into a clean 100-mL beaker. Add a stir bar.
5. Obtain a recent lot of Chlorine Solution Ampules, 50–70 mg/L, and note the actual free chlorine concentration for this lot.
6. Calculate the amount of Chlorine Solution to be added to the ammonia standard using the following equation:

$$\text{mL chlorine solution required} = \frac{455}{\text{free chlorine concentration}}$$

7. Open an ampule and use a glass Mohr pipet to add the calculated amount of Chlorine Solution slowly to the ammonia standard, while mixing at medium speed on a stir-plate.
8. Allow the monochloramine solution to mix for 1 minute after all Chlorine Solution is added.
9. Quantitatively transfer the monochloramine solution to a clean 100-mL Class A volumetric flask. Dilute to the mark with organic-free water, cap, and mix thoroughly. This is a nominal 4.5-mg/L (as Cl_2) monochloramine standard.
10. Use this standard within 1 hour of preparation. Analyze according to the Low Range Monochloramine procedure described above.
11. To adjust the calibration curve using the reading obtained with the 4.5-mg/L standard solution, select Options>More>Standard Adjust from the instrument menu.
12. Turn on the Standard Adjust feature and accept the shown concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
66	2.60 mg/L Cl_2	2.58–2.62 mg/L Cl_2	0.04 mg/L Cl_2

Summary of method

In the presence of a cyanoferrate catalyst, monochloramine (NH_2Cl) in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Cat. No.
Monochlor F Reagent Pillows	1	50/pkg	2802246

Recommended standards

Description	Unit	Cat. No.
Buffer Powder Pillows pH 8.3	25/pkg	89868
Chlorine Standard Solution,10-mL Voluette® Ampule 50–75 mg/L	16/pkg	1426810
Chlorine Standard Solution, 2-mL PourRite® Ampule 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 2-mL PourRite® Ampule 25–30 mg/L	20/pkg	2630020
Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N	500 mL	2406549
Nitrogen, Ammonia Standard Solution, 1000-mg/L as NH_3–N	1 L	2354153
Organic-free Water	500-mL	2641549

Optional reagents and apparatus

Description	Unit	Cat. No.
Beaker, glass, 100 mL	each	50042H
Flask, volumetric, Class A, 100-mL	each	1457442
Monochlor F Reagent Powder Pillows	100/pkg	2802299
Monochloramine/Free Ammonia Spec Check Kit	each	2507500
Pipet, Mohr, glass, 5-mL	each	2093437
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips for TenSette Pipet	50/pkg	2185696
Pipet Tips for TenSette Pipet	1000/pkg	2185628
Pipet, volumetric, Class A, 2-mL	each	1451536
Pipet, volumetric, Class A, 50-mL	each	1451541
pH Paper, 0–14 pH range	100/pkg	2601300
PourRite Ampule Breaker, for 2-mL ampules	each	2484600
Rochelle Salt Solution	29 mL DB	172533
Stir Bar, octagonal	each	2095352
Stirrer, magnetic	each	2881200
Shears	each	2369400
Thermometer, non-mercury, –10 to +225 °C	each	2635700
Voluette Ampule Breaker, for 10-mL ampules	each	2196800

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Edition 7

Chloride

DOC316.53.01017

Mercuric Thiocyanate Method

Method 8113

(0.1 to 25.0 mg/L Cl^-)

Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 77 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

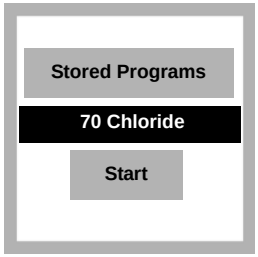
Filter turbid samples with moderately rapid filter paper and a funnel before analysis.
Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain.
Refer to the MSDS sheet for safe handling and disposal of hazardous waste. Gloves are recommended.

Collect the following items:

Description	Quantity
Ferric Ion Solution	1 mL
Mercuric Thiocyanate Solution	2 mL
Deionized Water	10 mL
Sample Cells (see *Instrument-specific information*)	2
Pipet, TenSette®, 0.1 to 1.0 mL	1
Pipet tips for 0.1 to 1.0 mL TenSette pipet	2

See *Consumables and replacement items* for reorder information.

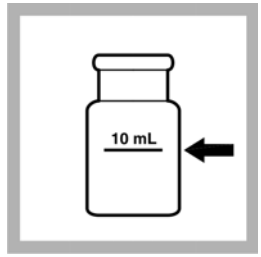
Mercuric Thiocyanate method for Chloride



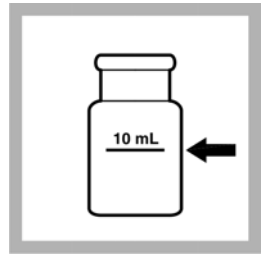
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

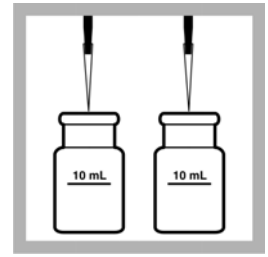
Refer to the user manual for orientation.



2. Prepared Sample: Fill a sample cell with 10 mL of sample.

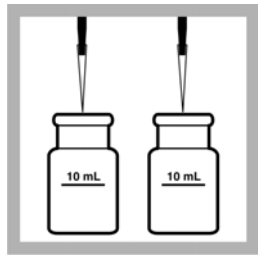


3. Blank Preparation: Fill another sample cell with 10 mL of deionized water.



4. Pipet 0.8 mL of Mercuric Thiocyanate Solution into each sample cell. ***Note: Use 1.0 mL with DR 5000***.

5. Swirl to mix.



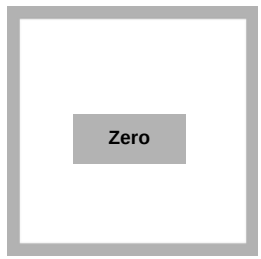
6. Pipet 0.4 mL of Ferric Ion Solution into each sample cell. ***Note: Use 0.5 mL with DR 5000***.

7. Swirl to mix. An orange color will develop if chloride is present.

8. Start the instrument timer.

A two-minute reaction time will begin.

9. Within five minutes after the timer expires, wipe the blank and insert it into the cell holder.

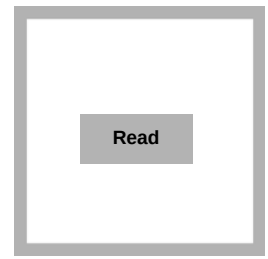


10. ZERO the instrument.

The display will show:

0.0 mg/L Cl^-

11. Wipe the prepared sample and insert it into the cell holder.



12. READ the results in mg/L Cl^-.

Interferences

Table 78 Interfering substances and levels

Interfering substance	Interference levels and treatments
Extreme pH	The pH should be about 2 after adding reagents. If the sample is strongly acidic or alkaline, adjust a portion of sample before testing to a pH of about 7. Use either 5.0 N Sodium Hydroxide Standard Solution[1] or a 1:5 dilution of perchloric acid. **Use pH paper**; most pH electrodes will contaminate the sample with chloride.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

Collect samples in glass or plastic containers. Samples can be stored for at least 28 days at room temperature.

Accuracy check

Required for accuracy check:

- Chloride Standard Solution, 1000-mg/L
- 50 mL mixing cylinders (3)
- TenSette Pipet and tips

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard additions from the instrument menu.
3. Default values for standard concentration, sample volume, and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare three sample spikes. Fill three 50 mL mixing cylinders with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of 1000-mg/L Chloride Standard Solution, respectively, to the cylinders and mix each thoroughly.
5. Analyze a 10 mL portion of each sample spike as described in the *Mercuric Thiocyanate method for Chloride* test, starting with the 0.1 mL sample spike. Each addition should reflect approximately 100% recovery.
6. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard Solution Method
Required for accuracy check:

- Chloride Standard Solution, 1000-mg/L
- Volumetric Flask, 500-mL
- Pipet, Class A, 10 mL

1. Prepare a 20.0-mg/L chloride standard solution.
   a. Pipet 10.00 mL of Chloride Standard Solution, 1000-mg/L, into a 500-mL volumetric flask.
   b. Dilute to the mark with deionized water. Follow the *Mercuric Thiocyanate method for Chloride* test.

2. To adjust the calibration curve using the reading obtained with the standard solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	Portion of curve
70	20.0 mg/L Cl^-	17.9–22.1 mg/L Cl^-	0.1 mg/L Cl^-	1.0 mg/L
			0.3 mg/L Cl^-	10.0 mg/L
			0.6 mg/L Cl^-	20.0 mg/L

Summary of method

Chloride in the sample reacts with mercuric thiocyanate to form mercuric chloride and liberate thiocyanate ion. Thiocyanate ions react with the ferric ions to form an orange ferric thiocyanate complex. The amount of this complex is proportional to the chloride concentration. Test results are measured at 455 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chloride Reagent Set, includes:	—	250 tests/pkg[1]	2319800
(1) Ferric Ion Solution	1 mL	100 mL	2212242
(1) Mercuric Thiocyanate Solution	2 mL	200 mL	2212129
Water, deionized	10 mL	4 L	27256

[1] This test requires a reagent blank. The number of tests shown refers to any combination of samples and blanks.

Required apparatus

Description	Quantity	Unit	Catalog number
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	varies	50/pkg	2185696
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Chloride Standard Solution, 1000-mg/L Cl^-	500 mL	18349

Optional reagents and apparatus

Description	Unit	Catalog number
Chloride Standard Solution, 10-mL Voluette® Ampule, 12,500-mg/L Cl^-	16/pkg	1425010
Chloride Standard Solution, 100-mg/L Cl^-	1 L	2370853
Cylinder, mixing, 50 mL	each	189641
Filter Paper, funnel, 125 mm	100/pkg	69257
Funnel, poly, 75 mm	each	108368
Gloves, chemical resistant, size 9–9.5[1]	pair	2410104
Perchloric Acid, ACS	680 g	75765
pH Paper, 1.0–11.0 pH range, 15 foot roll	5/pkg	39133
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet Filler, safety bulb	each	1465100
Sodium Hydroxide Standard Solution, 5.0 N	50 mL	245026
Voluette Ampule Breaker, for 10-mL ampules	each	2196800

[1] Other sizes available

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Edition 7

Chloride

DOC316.53.01170

Mercuric Nitrate Method **Method 8206**

10 to 8000 mg/L as Cl^- **Digital Titrator**

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

mg/L sodium chloride = mg/L chloride x 1.65
meq/L chloride = mg/L chloride / 35.45
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

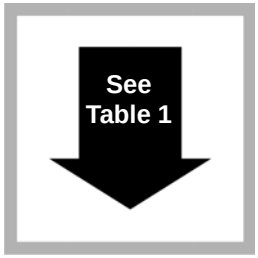
[1] See *Optional reagents and apparatus*.

Collect the following items:

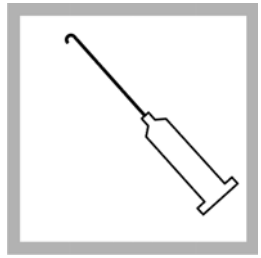
Description	Quantity
Diphenylcarbazone Indicator Powder Pillow	1 pillow
Mercuric Nitrate titration cartridge (see *Range-specific information*)	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Chloride



1. Select a sample volume and titration cartridge from the *Range-specific information* table.



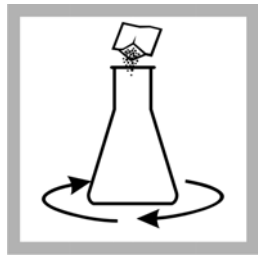
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table in a 250 mL Erlenmeyer flask.

Chloride (continued)

5. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.



6. Add the contents of one Diphenylcarbazone Indicator Powder Pillow. Swirl to mix.

Results will still be accurate if a small amount of powder does not dissolve.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from yellow to light pink.

Write down the number of digits displayed on the counter.

8. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L Cl^-

Example: 100 mL of sample was titrated with the 0.2256 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 mg/L Cl^-

Table 79 Range-specific information

Range (mg/L as Cl^-)	Sample volume (mL)	Titration cartridge (N $Hg(NO_3)_2$)	Multiplier
10–40	100	0.2256	0.1
40–160	25	0.2256	0.4
100–400	100	2.256	1.0
200–800	50	2.256	2.0
500–2000	20	2.256	5.0
1000–4000	10	2.256	10.0
2000–8000	5	2.256	20.0

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. The sample can be stored for up to 7 days before the analysis.

Interferences

Interfering substances lists substances that can interfere with this test.

Table 80 Interfering substances

Interfering substance	Interference level
Bromide	Interferes directly and is included in the test result.
Chromate	Concentrations above 10 mg/L interfere with this method.
Ferric iron	Concentrations above 10 mg/L interfere with this method.
Iodide	Interferes directly and is included in the test result.
pH	Neutralize strongly alkaline or acidic samples to a pH of 2 to 7 with 5.25 N sulfuric acid or 5.0 N sodium hydroxide. If a pH meter is used in the pH adjustment, use a separate sample to find the correct amount of acid or base to use. Then add the same amount of acid or base to the sample to be tested. pH electrodes will contaminate the sample.
Sulfide	Complete the following steps to remove sulfide interference: **1.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow to approximately 125 mL of sample. **2.** Mix for one minute. **3.** Filter through folded filter paper. **4.** Use the filtered sample in the chloride test procedure.
Sulfite	Concentrations above 10 mg/L interfere with this method. Eliminate sulfite interference by adding three drops of Hydrogen Peroxide, 30%, to the sample before the test is started.

Accuracy check

Use the standard additions method to find if the sample has an interference and to confirm analytical technique.

Standard additions method (sample spike)
Required for accuracy check:

- Chloride Voluette® Ampule Standard Solution, 12,500-mg/L Cl^-
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL

1. Open the standard solution ampule.

2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.

3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.

5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.

7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

8. Each 0.1 mL of standard that was added will use approximately 12.5 digits of the 2.256 N titration cartridge or 125 digits of the 0.2256 N titration cartridge to reach the endpoint.

If more or less titrant was used, the problem can be due to user technique, an interference (see *Interferences*) or a problem with reagents or apparatus.

Summary of method

When using Mercuric Nitrate Standard Solution, the sample is titrated under acidic conditions in the presence of diphenylcarbazone indicator. Upon addition of a slight excess of mercuric ion, a pink-purple complex is formed with the indicator, signaling the end point.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chloride Reagent Set (approximately 100 tests):			2272600
(2) Diphenylcarbazone Powder Pillows	1 pillow	100/pkg	83699
(1) Mercuric Nitrate Titration Cartridge, 0.2256 N	varies	each	1439301
(1) Mercuric Nitrate Titration Cartridge, 2.256 N	varies	each	92101

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Delivery tubes w/ 180° hook	1	each	1720500
Delivery tubes w/ 90° hook	1	each	4157800

Recommended standards

Description	Unit	Catalog number
Chloride Standard Solution, Voluette® Ampule, 12,500-mg/L Cl^-, 10-mL	16/pkg	1425010
Voluette breaker	1	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Filter paper, 12.5 cm	100/pkg	69257
Funnel, analytical, poly, 65 mm	each	108367
Hydrogen Peroxide, 30%, ACS	473 mL	14411
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
Sulfide Inhibitor Reagent Powder Pillow	100/pkg	241899
Sulfuric Acid Standard Solution, 5.25 N	100 mL MDB	244932
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
pH paper	—	2601300
pH Test Strip , 0–14 pH	100/pkg	2601300
Pipet tips	100/pkg	2185628
Pipet tips	50/pkg	2185696
Chloride standard solution, 1000 mg/L	500 mL	18349
Sampling bottle	250 mL	2087076
Clipper for Medium PWD PLWS	each	96800

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Edition 7

Chloride

DOC316.53.01171

Silver Nitrate Method **Method 8207**

10 to 10,000 mg/L as Cl^- **Digital Titrator**

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

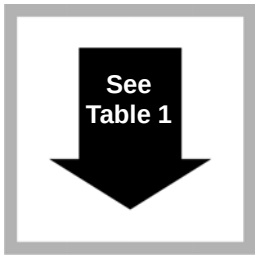
mg/L sodium chloride = mg/L chloride x 1.65
meq/L chloride = mg/L chloride / 35.45
For added convenience when stirring, use the TitraStir® stirring apparatus.

Collect the following items:

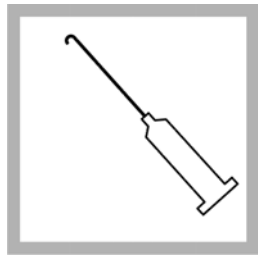
Description	Quantity
Chloride 2 Indicator Powder Pillow	1 pillow
Silver Nitrate titration cartridge (see *Range-specific information*)	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Chloride



1. Select a sample volume and titration cartridge from the *Range-specific information* table.



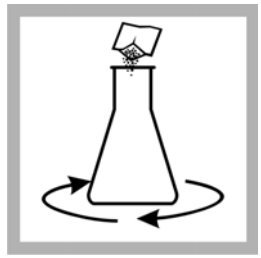
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table into a 250 mL Erlenmeyer flask.

Chloride (continued)

5. Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.



6. Add the contents of one Chloride 2 Indicator Powder Pillow. Swirl to mix.

Results will still be accurate if a small amount of powder does not dissolve.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from yellow to red-brown.

Write down the number of digits displayed on the counter.

8. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L Cl^-

Example: 100 mL of sample was titrated with the 0.2256 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 mg/L Cl^-

Table 81 Range-specific information

Range (mg/L as Cl^-)	Sample volume (mL)	Titration cartridge (N $AgNO_3$)	Multiplier
10–40	100	0.2256	0.1
25–100	40	0.2256	0.25
100–400	50	1.128	1.0
250–1000	20	1.128	2.5
1000–4000	5	1.128	10.0
2500–10,000	2	1.128	25.0

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. The sample can be stored for up to 7 days before the analysis.

Interferences

Interfering substances lists substances that can interfere with this test.

Table 82 Interfering substances

Interfering substance	Interference level
Bromide	Interferes directly and is included in the test result.
Cyanide	Interferes directly and is included in the test result.
Iron	Concentrations above 10 mg/L mask the end point.

Table 82 Interfering substances (continued)

Interfering substance	Interference level
Iodide	Interferes directly and is included in the test result.
Orthophosphate	Concentrations above 25 mg/L will precipitate the silver.
pH	Neutralize strongly alkaline or acidic samples to a pH of 2 to 7 with 5.25 N sulfuric acid or 5.0 N sodium hydroxide. If a pH meter is used in the pH adjustment, use a separate sample to find the correct amount of acid or base to use. Then add the same amount of acid or base to the sample to be tested. pH electrodes will contaminate the sample.
Sulfide	Complete the following steps to remove sulfide interference: **1.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow to approximately 125 mL of sample. **2.** Mix for one minute. **3.** Filter through folded filter paper. **4.** Use the filtered sample in the chloride test procedure.
Sulfite	Concentrations above 10 mg/L interfere with this method. Eliminate sulfite interference by adding three drops of Hydrogen Peroxide, 30%, to the sample before the test is started.

Accuracy check

Use the standard additions method to determine whether the sample has an interference and confirm analytical technique.

Standard additions method (sample spike)
Required for accuracy check:

- Chloride Voluette® Ampule Standard Solution, 12,500-mg/L Cl^-
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL

1. Open the standard solution ampule.

2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.

3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.

5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.

7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

8. Each 0.1 mL of standard that was added will use approximately 12.5 digits of the 2.256 N titration cartridge or 25 digits of the 1.128 N titration cartridge to reach the endpoint.

If more or less titrant was used, the problem can be due to user technique, an interference (see *Interferences*) or a problem with reagents or apparatus.

Summary of method

The sample is titrated with Silver Nitrate Standard Solution in the presence of potassium chromate (from the Chloride 2 Indicator Powder). The silver nitrate reacts with the chloride present to produce insoluble white silver chloride. After all the chloride has been precipitated, the silver ions react with the excess chromate present to form a red-brown silver chromate precipitate, marking the end point of the titration.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chloride Reagent Set (approximately 100 tests):			2288000
(2) Chloride 2 Indicator Powder Pillows	1 pillow	50/pkg	105766
(1) Silver Nitrate Titration Cartridge, 0.2256 N	varies	each	1439601
(1) Silver Nitrate Titration Cartridge, 1.128 N	varies	each	1439701

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Delivery tubes w/ 180° hook	1	each	1720500
Delivery tubes w/ 90° hook	1	each	4157800

Recommended standards

Description	Unit	Catalog number
Chloride Standard Solution, Voluette® Ampule, 12,500-mg/L Cl^-, 10-mL	16/pkg	1425010
Voluette breaker	—	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Filter paper, 12.5 cm	100/pkg	69257
Funnel, analytical, poly, 65 mm	each	108367
Hydrogen Peroxide, 30%, ACS	473 mL	14411
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
Sulfide Inhibitor Reagent Powder Pillow	100/pkg	241899
Sulfuric Acid Standard Solution, 5.25 N	100 mL MDB	244932
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
pH Test Strip, 0–14 pH	100/pkg	2601300
Pipet tips	100/pkg	2185628
Pipet tips	50/pkg	2185696
Chloride standard solution, 1000 mg/L	500 mL	18349
Sampling bottle	250 mL	2087076
Dropper, glass	5/pkg	1419705

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Edition 7

Chloride

DOC316.53.01153

USEPA[1] Silver Nitrate Buret Titration Method[2]

Method 8225

0 to 25,000 mg/L as Cl^-

Buret Titration

Scope and Application: For water, wastewater and seawater.

[1] USEPA accepted for NPDES reporting when 0.0141 N silver nitrate standard solution is used.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater, (Standard Method 4500 Cl^- B).*

Test preparation

Before starting the test:

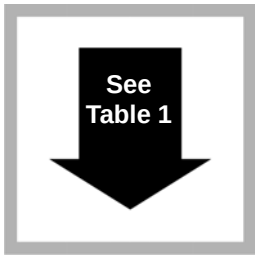
Adjust highly acidic or alkaline samples to a pH between pH 7 and 9. Use pH paper to measure the pH. A pH meter will contaminate the sample.
To calculate the result as mg/L sodium chloride (NaCl): mg/L chloride x 1.65 = mg/L sodium chloride
A small amount of silver nitrate is used to make the red-brown color in step 6. For most accurate results, follow the procedure using 100 mL of deionized water in place of the sample. Titrate this solution and note the volume of titrant required. For all samples, subtract this volume of titrant before calculating the mg/L chloride.

Collect the following items:

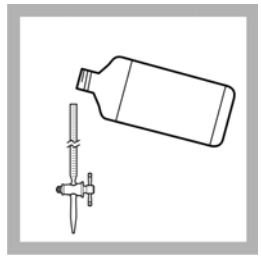
Description	Quantity
Chloride 2 Indicator Powder Pillow	1
Silver Nitrate Standard Solution (see *Range-specific information*)	1 bottle
Buret, Class A, 25-mL, with support stand	1
Erlenmeyer flask, 250 mL	1
Graduated cylinder	1

See *Consumables and replacement items* for reorder information.

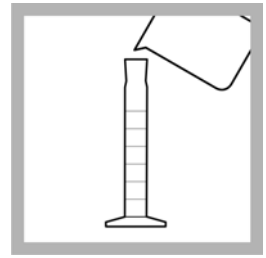
Buret titration



1. Select the sample volume and silver nitrate standard solution from *Range-specific information*.



2. Fill a 25-mL buret to the zero mark with the Silver Nitrate Standard Solution.



3. Use a graduated cylinder or pipet to measure the sample volume from *Range-specific information*.

4. Transfer the sample into a 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.

Buret titration (continued)

5. Add the contents of one Chloride 2 Indicator Powder Pillow. Swirl to mix.

6. Titrate the sample while swirling the flask until the color changes from yellow to red-brown.

7. Calculate:

mL titrant used x multiplier = mg/L chloride as Cl^-

Example: 100 mL of sample was titrated with the 0.0141 N silver nitrate solution and 15 mL of titrant was used to reach the endpoint.
The chloride concentration is: 15 x 5 = 75 mg/L as Cl^-

Table 83 Range-specific information

Range (mg/L as Cl^-)	Sample volume (mL)	Silver nitrate concentration	Multiplier
0–125	100	0.0141 N	5
100–250	50	0.0141 N	10
200–500	25	0.0141 N	20
500–1250	100	0.141 N	50
1000–2500	50	0.141 N	100
2500–10,000	25	0.141 N	200
5000–25,000	10	0.141 N	500

Interferences

An interfering substance can mask the end point. A dilution can reduce the interference to a level at which the substance does not interfere. If an interference is suspected, use a smaller amount of fresh sample and repeat the test.

Interfering substances lists substances that can interfere with this test.

Table 84 Interfering substances

Interfering substance	Interference level
Bromide	Cyanide, bromide and iodide interfere directly and are titrated as chloride.
Cyanide	Cyanide, bromide and iodide interfere directly and are titrated as chloride.
Iodide	Cyanide, bromide and iodide interfere directly and are titrated as chloride.
Iron	Iron concentrations over 20 mg/L will mask the end point.
Orthophosphate	Orthophosphate concentrations over 25 mg/L will cause a precipitate to form.
Sulfide	To remove interference from sulfide, add one Sulfide Inhibitor Reagent Powder Pillow to approximately 125 mL of the sample, mix for one minute and filter through filter paper.
Sulfite	To remove interference from at least 10 mg/L sulfite, add 3 drops of 30% hydrogen peroxide to 100 mL of sample before starting the test.

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Samples can be stored in sealed containers.

Accuracy check

Use the standard additions method to find if the sample has an interference. Use the standard solution method to make sure that the user has followed the test correctly and that the reagents are good.

Standard additions method (sample spike)
Required for accuracy check:

- Chloride Voluette® Ampule Standard, 12,500-mg/L as Cl^-
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL

Procedure for use with the **0.0141 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 0.1 mL of standard that was added should use 2.5 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Procedure for use with the **0.141 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 1.0 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 2.0 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 3.0 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 1.0 mL of standard that was added should use 2.5 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Standard solution method
A silver nitrate standard solution will slowly decompose with exposure to light. Complete the following test to make sure the concentration is accurate.

Required for accuracy check:

- Sodium Chloride Standard Solution, 1000-mg/L as Cl^-
- 100-mL Class A volumetric flask (for use with 0.0141 N titrant only)

Procedure for use with the **0.0141 N** titrant:

1. Add 10.0 mL of the sodium chloride standard solution, 1000-mg/L as Cl^-, to a 100-mL Class A volumetric flask. Dilute to 100 mL with deionized water and mix fully. This solution has a concentration of 100 mg/L chloride.
1. Add 100.0 mL of the diluted sodium chloride standard solution, 100-mg/L as Cl^-, to an Erlenmeyer flask.
2. Add the Chloride 2 indicator and swirl to mix.
3. Titrate the standard to the end point with the 0.0141 N silver nitrate titrant and calculate the result. If the result is more than 105 mg/L chloride, discard the silver nitrate titrant and replace it with a fresh supply.

Procedure for use with the **0.141 N** titrant:

1. Add 100.0 mL of the sodium chloride standard solution, 1000-mg/L as Cl^-, to an Erlenmeyer flask.
2. Add the Chloride 2 indicator and swirl to mix.
3. Titrate the standard to the end point with the 0.141 N silver nitrate titrant and calculate the result. If the result is more than 1050 mg/L chloride, discard the silver nitrate titrant and replace it with a fresh supply.

Summary of method

Silver nitrate is used as the titrant and potassium chromate as the indicator. Silver nitrate first reacts selectively with the chloride in the sample to produce insoluble white silver chloride. After all the chloride has been precipitated, the silver nitrate reacts with the chromate to form an orange or red-brown silver chromate precipitate.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chloride 2 Indicator Powder Pillows	1 pillow	50/pkg	105766
Titrant—select one or more based on range:			
Silver Nitrate Standard Solution, 0.0141 N	varies	1 L	31653
Silver Nitrate Standard Solution, 0.141 N	varies	500 mL	1255149

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Support Stand	1	each	56300

Recommended standards

Description	Unit	Catalog number
Chloride Standard Solution, Voluette® Ampule, 12,500-mg/L as Cl^-, 10-mL	16/pkg	1425010
Sodium Chloride Standard Solution, 1000-mg/L as Cl^-	500 mL	18349
Voluette Ampule breaker, 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Filter Paper, 12.5 cm diameter	100/pkg	69257
Hydrogen Peroxide, 30%	473 mL	14411
Sulfide Inhibitor Reagent Powder Pillow	100/pkg	241899
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Water, deionized	500 mL	27249
Pipet, TenSette®, Pipet, 1.0–10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010[1]	50/pkg	2199796
Pipet Tips, for TenSette Pipet 1970010[1]	250/pkg	2199725
Pipet, TenSette, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
pH Paper, 0–14 pH range	100/pkg	2601300
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Flask, Class A volumetric, 100 mL	each	1457442
Dropper, glass	5/pkg	1419705
Clippers for powder pillows	each	66800

[1] Other sizes are available

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Edition 7

Chlorine Dioxide

DOC316.53.01021

DPD Method[1] **Method 10126**

(0.04 to 5.00 mg/L) **Powder Pillows and AccuVac® Ampuls**

Scope and Application: For water and wastewater. USEPA accepted for reporting for drinking water analysis.[2]

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

[2] Procedure is equivalent to *Standard Methods, 18 ed., 4500 ClO_2 D.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 85 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

Analyze samples immediately because chlorine dioxide is unstable and volatile. See *Sample collection, preservation and storage*.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
After adding the DPD Free Chlorine Powder Pillow to the sample, a pink color will develop if chlorine dioxide is present.
If the chlorine dioxide concentration in the sample exceeds the upper limit of the test, the color may fade or the sample may turn yellow. Dilute the sample with high quality water that is chlorine demand-free, and repeat the test. Some loss of chlorine dioxide may occur. Multiply the result by the appropriate dilution factor.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
DPD Free Chlorine powder pillow, 10-mL	1
Glycine Reagent	4 drops
Sample cells (see *Instrument-specific information*)	2
Stopper for 18 mm tube	2
AccuVac Test:	
DPD Free Chlorine Reagent AccuVac® Ampuls	1
Glycine Reagent	16 drops
Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1
Stopper for 18 mm tube	1

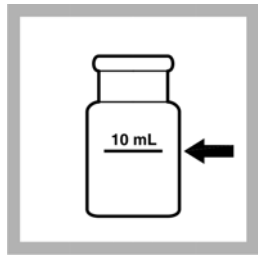
See *Consumables and replacement items* for reorder information.

DPD method, powder pillows

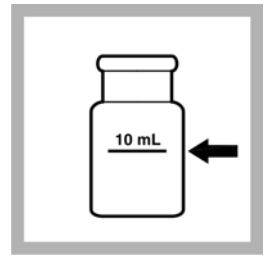
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



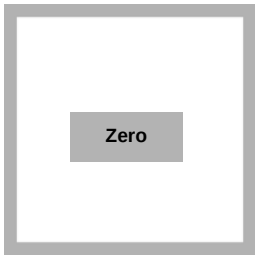
2. Blank Preparation: Fill a sample cell with 10 mL of sample. Close the sample cell.



3. Prepared Sample: Fill a second sample cell with 10 mL of sample. Close the sample cell.

4. Wipe the blank and insert it into the cell holder.

DPD method, powder pillows (continued)



5. **ZERO** the instrument.

The display will show: 0.00 mg/L ClO_2

6. Add four drops of Glycine Reagent to the sample. Swirl to mix.

7. Add the contents of one DPD Free Chlorine Powder Pillow to the prepared sample cell.

Swirl the sample for 20 seconds to mix.

8. Wait 30 seconds for any undissolved powder to settle.

Immediately proceed to step 9.

9. Within one minute of adding the DPD reagent, wipe the sample cell and insert it into the cell holder.

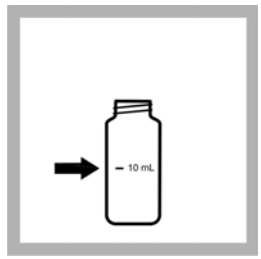
10. **READ** the results in mg/L ClO_2.

DPD method, AccuVac® Ampuls

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. **Blank Preparation:** Fill a round sample cell with 10-mL of sample.

3. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show: 0.00 mg/L ClO_2

4. **Prepared Sample:** Fill a 50-mL beaker with 40 mL of sample.

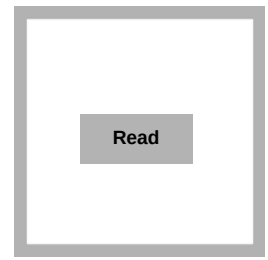
Add 16 drops of Glycine Reagent to the sample in the beaker. Swirl gently to mix.

DPD method, AccuVac® Ampuls (continued)

5. Fill a DPD Free Chlorine Reagent AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

6. Quickly invert the Ampul several times to mix. Wait 30 seconds for any undissolved powder to settle.

7. Within one minute of adding the sample, wipe the Ampul and insert it into the cell holder.



8. **READ** the results in mg/L ClO_2.

Interferences

Table 86 Interfering substances

Interfering substance	Interference level
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide[1]. Determine amount to be added on a separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 250 mg/L $CaCO_3$. Color may not develop fully or may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. Determine amount to be added on a separate sample aliquot, then add the same amount to the sample being tested. Correct for the volume addition.
Bromine, Br_2	Interferes at all levels.
Chlorine, Cl_2	May interfere at levels greater than 6 mg/L. Additional glycine may be able to compensate for this interference.
Chloramines, organic	May interfere.
Flocculating agents	High levels of most flocculating agents can be tolerated. This tolerance is decreased if chlorine is present. See the information about metals in this table. In the presence of 0.6 mg/L Cl_2, $Al(SO_4)_3$ (< 500 mg/L) and $FeCl_2$ (<200 mg/L) may be tolerated.
Hardness	No effect at less than 1000 mg/L as $CaCO_3$.
Iodine, I_2	Interferes at all levels.
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	Oxidized manganese interferes at all levels. Oxidized chromium interferes at levels greater than 2 mg/L. To remove the interferences: **5.** Adjust sample pH to 6–7. **6.** Add 3 drops Potassium Iodide[1] (30 g/L) to a 25-mL sample. **7.** Mix and wait one minute. **8.** Add 3 drops Sodium Arsenite[1, 2] (5 g/L) and mix. **9.** Analyze 10 mL of the treated sample as described in the procedure. **10.** Subtract the result of this test from the original analysis to obtain the correct chlorine dioxide concentration.

Table 86 Interfering substances (continued)

Interfering substance	Interference level
Metals	Various metals may interfere by combining with the glycine needed to remove the chlorine interference. Metal interference is limited except when chlorine is present. In the presence of 0.6 mg/L Cl_2, both copper (>10 mg/L) and nickel (>50 mg/L) interfere. Other metals may also interfere, depending on their ability to prevent glycine from reacting with any Cl_2 in the sample. It may be necessary to add more glycine to overcome this interference.
Monochloramine	Causes a gradual drift to higher readings. When read within 1 minute after reagent addition, 3 mg/L monochloramine causes less than a 0.1 mg/L ClO_2 increase in the reading.
Ozone	Interferes at levels greater than 1.5 mg/L.
Peroxides	May interfere.
Extreme sample pH	Adjust to pH 6–7.
Highly buffered samples	Adjust to pH 6–7.

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). Refer to a current MSDS for proper disposal instructions.

Sample collection, preservation and storage

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine dioxide analysis immediately.

Accuracy check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. The manufacturer does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, Part 4500-ClO_2 Chlorine Dioxide, under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution". Prepare a chlorine dioxide standard.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
76	DR 5000	3.00 mg/L ClO_2	2.89–3.11 mg/L ClO_2	0.04 mg/L ClO_2
77	DR 5000	3.00 mg/L ClO_2	2.91–3.09 mg/L ClO_2	0.04 mg/L ClO_2

Summary of method

Chlorine dioxide reacts with DPD (N, N-diethyl-p-phenylenediamine) to the extent of one-fifth of its total available chlorine content, corresponding to reduction of chlorine dioxide to chlorite. The resulting pink color intensity is proportional to the ClO_2 in the sample. Chlorine interference is eliminated by adding glycine, which converts free chlorine to chloroaminoacetic acid, but has no effect on chlorine dioxide at the test pH. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chlorine Dioxide DPD/Glycine Reagent Set (100 tests), includes:			2770900
(1) DPD Free Chlorine Reagent Powder Pillows, 10-mL	1	100/pkg	2105569
(1) Glycine Reagent	4 drops	29 mL	2762133
OR			
Chlorine Dioxide DPD/Glycine AccuVac® Ampul Reagent Set (25 tests), includes:			2771000
(1) DPD Free Chlorine Reagent AccuVac® Ampuls	1	25/pkg	2502025
(1) Glycine Reagent	16 drops	29 mL	2762133

Required apparatus

Description	Quantity	Unit	Catalog number
AccuVac Snapper	1	each	2405200
Beaker, 50-mL	1	each	50041H
Stopper for 18 mm tube	1	6/pkg	173106
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
Voluette Ampule Breaker, for 10-mL ampules	each	2196800
Water, organic-free	500 mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
AccuVac Vials, for sample blanks	25/pkg	2677925
DPD Free Chlorine Reagent Powder Pillows, 10-mL	1000/pkg	2105528
DPD Free Chlorine Reagent Powder Pillows, 10-mL	300/pkg	2105503
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sodium Hydroxide, 1 N	100 mL	104532
Standard Methods Book, most current edition	each	2270800
Stopper for 18 mm tube	25/pkg	173125
Sulfuric Acid, 1 N, 100 mL	each	127032

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Edition 7

Chlorine Dioxide

DOC316.53.01019

Direct Reading Method **Method 8138**

HR (5 to 1000 mg/L)

Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 87 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Chlorine dioxide is unstable and volatile. Analyze samples immediately.
Gloves and goggles are recommended.

Collect the following items:

Description	Quantity
Water, deionized	10 mL
Sample cell (see *Instrument-specific information*)	2

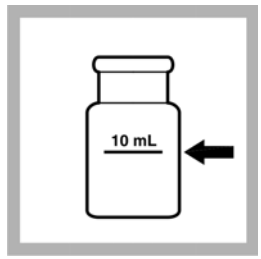
See *Consumables and replacement items* for reorder information.

Direct Reading method

1. Select the test.

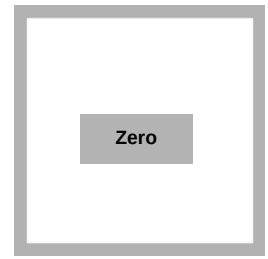
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a sample cell to the 10-mL mark with deionized water.

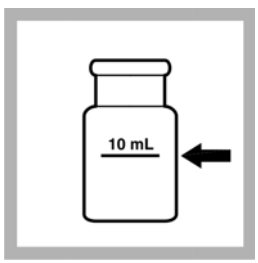
3. Wipe the blank and insert it into the cell holder.



4. ZERO the instrument.

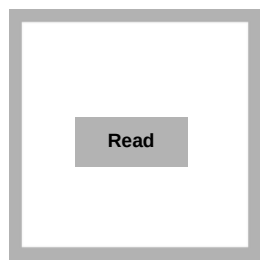
The display will show:

0 mg/L ClO_2



5. Prepared Sample: Fill a second sample cell to the 10-mL mark with sample.

6. Wipe the prepared sample and insert it into the cell holder.



7. READ the results in mg/L ClO_2.

Interferences

Because this is a Direct Reading test, no known interferences exist.

Sample collection, storage and preservation

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no

headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine dioxide analysis immediately.

Accuracy check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. The manufacturer does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, Part 4500-ClO_2 Chlorine Dioxide, under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution". Prepare a 500-mg/L chlorine dioxide standard.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
75	469 mg/L ClO_2	459–479 mg/L ClO_2	5 mg/L ClO_2

Summary of method

Chlorine dioxide, a yellow gas, can be measured directly in a water solution. Test results are measured at 445 nm.

Consumables and replacement items

Required reagents and apparatus

Description	Quantity/Test	Unit	Catalog number
Water, deionized	10 mL	4 L	27256
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional apparatus

Description	Unit	Catalog number
Gloves, chemical resistant, size 9–9.5[1]	pair	2410104
Safety Goggles, vented	each	2550700
Standard Methods Book, most current edition	each	2270800

[1] Other sizes available.

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Edition 7

Chlorine Dioxide

DOC316.53.01022

Chlorophenol Red Method[1]

Method 8065

LR (0.01 to 1.00 mg/L)

Scope and Application: For water and wastewater

[1] Adapted from Harp, Klein and Schoonover, *Jour. Amer. Water Works Assn.*, 73 387–388 (1981).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 88 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Chlorine dioxide is unstable and volatile. Analyze samples immediately.
For most accurate results, analyze each portion of sample at the same temperature.
A TenSette® Pipet may be used to dispense Chlorine Dioxide Reagent 1 and Chlorine Dioxide Reagent 3

Collect the following items:

Description	Quantity
Chlorine Dioxide Reagent 1	2 mL
Chlorine Dioxide Reagent 2	2 mL
Chlorine Dioxide Reagent 3	2 mL
Dechlorinating Reagent Pillows	1
Cylinder, graduated mixing, 50 mL	2
Pipet, volumetric, Class A, 1 mL	3
Pipet Filler, with safety bulb	1
Sample Cells (see *Instrument-specific information*)	2

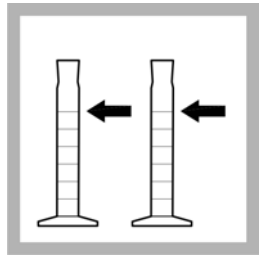
See *Consumables and replacement items* for reorder information.

Powder Pillows

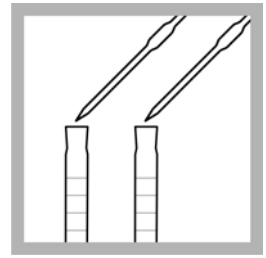
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

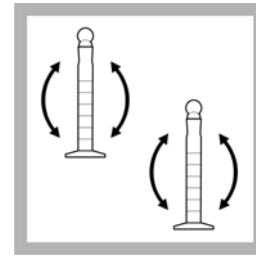
Refer to the user manual for orientation.



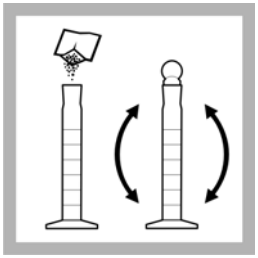
2. Fill two 50-mL mixing cylinders to the 50-mL mark with sample.



3. Use a volumetric pipet and pipet filler to add 1.0 mL of Chlorine Dioxide Reagent 1 to each cylinder. Stopper.



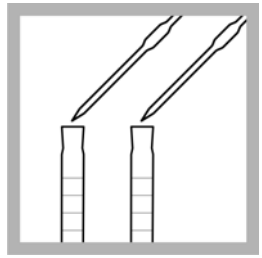
4. Invert several times to mix.



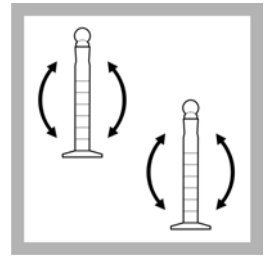
5. Blank Preparation: Add the contents of one Dechlorinating Reagent Powder Pillow to one cylinder. (This is the blank).

Stopper and invert several times until dissolved.

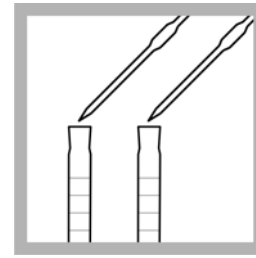
The second cylinder, which does **not** receive dechlorinating reagent, is the prepared sample.



6. Use a volumetric pipet to add exactly 1.00 mL of Chlorine Dioxide Reagent 2 to each cylinder. Stopper.

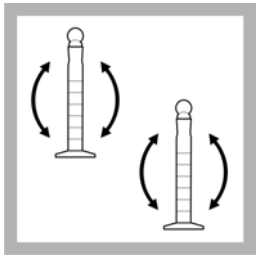


7. Invert several times to mix.

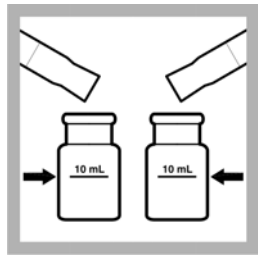


8. Use a volumetric pipet and pipet filler to add 1.0 mL of Chlorine Dioxide Reagent 3 to each cylinder. Stopper.

Powder Pillows



9. Invert several times to mix.



10. Pour 10 mL from each cylinder into two sample cells.

11. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show: 0.00 mg/L ClO_2

12. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L ClO_2.

Interferences

Table 89 Interfering substances and levels

Interfering substance	Interference levels and treatments
Highly acidic or alkaline water	May require 2.0 mL each of Chlorine Dioxide Reagent 1 and Chlorine Dioxide Reagent 3 instead of 1.0 mL
ClO^-	Greater than 5.5 mg/L
ClO_2^-	Greater than 6 mg/L
ClO_3^-	Greater than 6 mg/L
CrO_4^{2-}	Greater than 3.6 mg/L
Fe^{3+}	Greater than 5 mg/L
Hardness	Greater than 1000 mg/L
Ozone	Greater than 0.5 mg/L
Turbidity	Greater than 1000 NTU

Sample collection, preservation and storage

- Analyze samples for chlorine dioxide immediately after collection.
- Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine dioxide in water.
- Avoid plastic containers since these may have a large chlorine dioxide demand.
- Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

- A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample.

Accuracy check

Standard solution method
Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. The manufacturer does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, Part 4500-ClO_2 Chlorine Dioxide, under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution". Prepare a 0.50-mg/L chlorine dioxide standard.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
72	0.53 mg/L ClO_2	0.50–0.55 mg/L ClO_2	0.01 mg/L ClO_2

Summary of method

Chlorine Dioxide (ClO_2) is determined by its combination with chlorophenol red at pH 5.2 to form a colorless complex. The net effect is bleaching of the color in an amount proportional to the chlorine dioxide concentration. The method is specific for ClO_2 and is unreactive to other active chlorine or moderate oxidizing compounds. Test results are measured at 575 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chlorine Dioxide Reagent Set (100 Tests), includes:	—	each	2242300
(2) Chlorine Dioxide Reagent 1	2 mL	100 mL	2070042
(2) Chlorine Dioxide Reagent 2	2 mL	100 mL	2070142
(2) Chlorine Dioxide Reagent 3	2 mL	100 mL	2070242
(1) Dechlorinating Reagent Powder Pillows	1	100/pkg	1436369

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated mixing, 50-mL	2	each	189641
Pipet, volumetric, Class A, 1.00-mL	3	each	1451535
Pipet Filler, safety bulb	1	each	1465100
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Standard Methods book, current edition	each	2270800
pH Paper, 0–14 pH range	100/pkg	2601300

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Edition 7

Chlorine Dioxide

DOC356.53.01018

Amaranth Method[1]

(20 to 500 µg/L)

Scope and Application: For water, drinking water

[1] This method is under license of Elf Atofina. Reagent sets for this method are only available in Europe.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 90 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

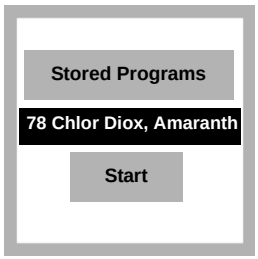
Chlorine dioxide is unstable and volatile. Analyze samples immediately. See *Sample collection, preservation and storage*.
For most accurate results, analyze each portion of sample at the same temperature.
For best precision, measurement of the reagent with a volumetric pipet or high precision pipettor is recommended.
A TenSette® Pipet may be used to dispense Chlorine Dioxide Reagent A.

Collect the following items:

Description	Quantity
Chlorine Dioxide Reagent Set	1
Volumetric Flask, 25-mL plastic	2
Syringe, 1-mL with needle	1
Sample cell (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

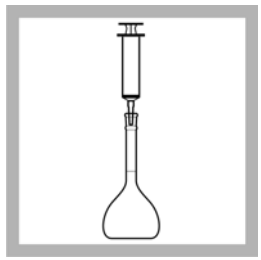
Amaranth method



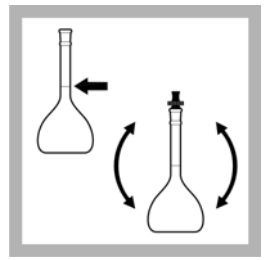
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

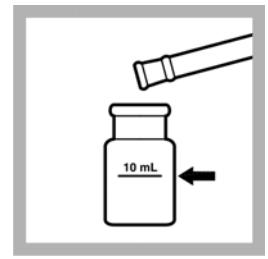
Refer to the user manual for orientation.



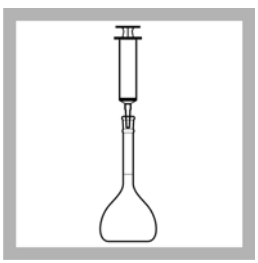
2. Blank Preparation: Using the syringe and needle provided, add 1.0 mL of Chlorine Dioxide Reagent A into a 25-mL volumetric flask.



3. Fill the volumetric flask to the mark with deionized water. Stopper. Invert at least 7 times to mix.

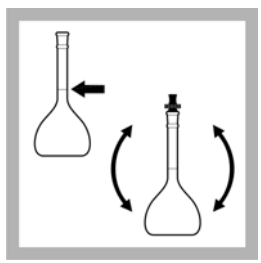


4. Pour 10 mL from the volumetric flask into a 10 mL sample cell.



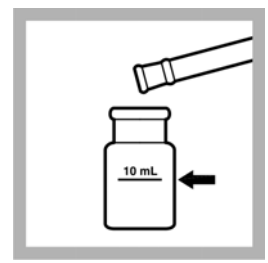
5. Prepared Sample: Add 1.0 mL of Chlorine Dioxide Reagent A into a second 25-mL volumetric flask.

Use a volumetric pipet and pipet filler or a TenSette Pipet to add this reagent.



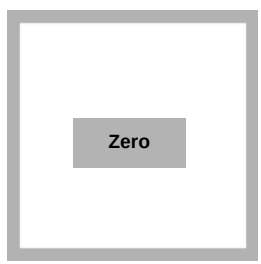
6. Fill the second volumetric flask to the mark with the sample. Stopper. Invert at least 7 times to mix.

7. Start the instrument timer. A 1-minute reaction period will begin.



8. Prepared Sample: Pour 10 mL from the second volumetric flask into a second sample cell.

9. Wipe the blank and insert it into the cell holder.

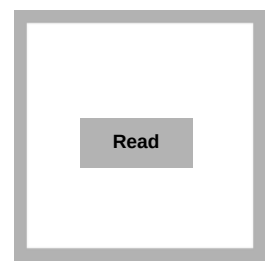


10. ZERO the instrument.

The display will show:

0 µg/L ClO_2

11. When the timer expires, wipe the prepared sample and insert it into the cell holder.



12. READ the results in µg/L ClO_2.

Interferences

Table 91 Interfering substances and levels

Interfering substance	Interference level
ClO^-	Greater than 2.0 mg/L
ClO_2^-	Greater than 2.0 mg/L
ClO_3^-	Greater than 2.0 mg/L
CrO_4^{2-}	Greater than 0.2 mg/L
Fe^{3+}	Greater than 0.5 mg/L
Hardness	Greater than 1000 mg/L
Ozone	Greater than 0.5 mg/L
Turbidity	Greater than 1000 NTU

Sample collection, preservation and storage

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the analysis immediately.

Accuracy check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. The manufacturer does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, Part 4500-ClO_2 Chlorine Dioxide, under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution". Prepare a 0.25-mg/L (250-µg/L) chlorine dioxide standard.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
78	250 µg/L ClO_2	192–308 µg/L ClO_2	24 µg/L ClO_2

Summary of method

Chlorine dioxide (ClO_2) is determined by its combination with Amaranth. Color intensity decreases as the level of chlorine dioxide increases. Test results are measured at 521 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chlorine Dioxide Reagent Set (100 Tests)[1]	1	100/pkg	LYW240

[1] Available only in Europe.

Required apparatus

Description	Quantity	Unit	Catalog number
Chlorine Dioxide Tool Set[1], includes:	—	each	LZC140
(2) Flask, volumetric, 25-mL	2	each	—
(1) Syringe, 1-mL, with needle	1	each	—

[1] Available only in Europe.

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2815696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet Filler, safety bulb	each	1465100
Pipet, volumetric, Class A, 1.00-mL	each	1451535
Standard Methods Book (most current edition)	each	2270800
Water, organic-free	500 mL	2641549

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Edition 7

Chlorine Dioxide

DOC316.53.01020

Direct Reading Method **Method 8345**

MR (1 to 50 mg/L)

Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 92 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800	2495402	Fill line faces right

Before starting the test:

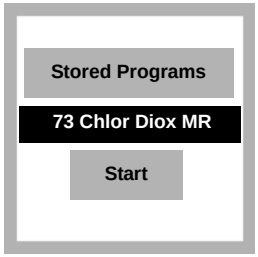
Chlorine dioxide is unstable and volatile. Analyze samples immediately.
Gloves and goggles are recommended.

Collect the following items:

Description	Quantity
Water, deionized	10 mL
Sample cell (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

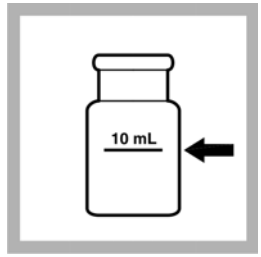
Direct Reading method



1. Select the test.

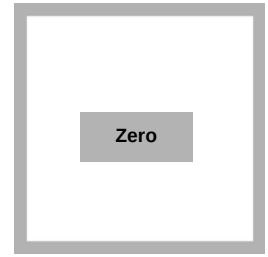
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a sample cell to the 10-mL mark with deionized water.

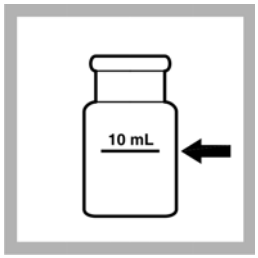
3. Wipe the blank and insert it into the cell holder.



4. ZERO the instrument.

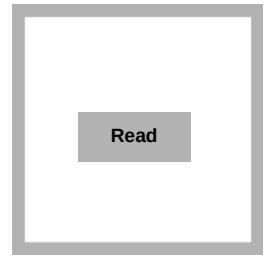
The display will show:

0 mg/L ClO_2



5. Prepared Sample: Fill a second sample cell to the 10-mL mark with sample.

6. Wipe the prepared sample and insert it into the cell holder.



7. READ the results in mg/L ClO_2.

Sample collection, preservation and storage

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine dioxide analysis immediately.

Accuracy check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. The manufacturer does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, see the instructions in *Standard Methods for the Examination of Water and Wastewater*, Part 4500-ClO_2 Chlorine Dioxide, under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution". Prepare a 25.0-mg/L chlorine dioxide standard.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
75	43 mg/L ClO_2	41–45 mg/L ClO_2	0.3 mg/L ClO_2

Summary of method

Chlorine dioxide, a yellow gas, can be measured directly in a water solution. Test results are measured at 360 nm.

Consumables and replacement items

Required reagents and apparatus

Description	Quantity/Test	Unit	Catalog number
Water, deionized	10 mL	4 L	27256
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional apparatus

Description	Unit	Catalog number
Gloves, chemical resistant, size 9–9.5[1]	pair	2410104
Safety Goggles, vented	each	2550700
Standard Methods Book, most current edition	each	2270800

[1] Other sizes available.

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Edition 7

Chlorine Demand/Requirement

DOC316.53.01146

Method 10223

DPD Reagent [1]

Scope and Application: For determining the chlorine demand and the chlorine requirement in drinking water production. For establishing chlorine demand constants and establishing historical background data on raw water quality. For determining chlorine demand on distributed waters.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater, Section 2350*

Test preparation

Before starting the test:

Important Note: *Read Getting started and all procedure steps before performing this test.*
Develop a chlorine demand plan to detail the number of sample doses, the concentration of chlorine dose additions and length of chlorine contact time. See *Chlorine demand test plan* and *Getting started*.
Precondition sample containers, test bottles and labware to be chlorine demand free. See *Treatment of analysis labware*.
Allow time for samples to equilibrate to the temperature indicated in the test plan before beginning the test.

Collect the following items:

Description	Quantity
DPD Free Chlorine Reagent PP, 10-mL or 25-mL	Varies
Chlorine Dosing Solution Ampules	Varies
Sample Bottles and Caps	6
Bottle Labels	6
pH Meter	1
Thermometer	1
Pipet, TenSette®, 0.1–1.0 mL and tips	1
Stir Plate	1
Stir Bar Magnets	6
Sample Cells, 10 mL 1-inch square or 1-cm/10 mL	2
Spectrophotometer or Colorimeter	1

DPD Reagent

1. Complete a *Chlorine demand test plan*.

Measure and record the temperature and pH of the sample water to be tested.

2. Prepare 6 chlorine demand-free bottles. Rinse each bottle with sample and fill each 118-mL bottle with approximately 100 mL of the sample to be tested.

Label the bottles 1 to 6.

3. Use tweezers or tongs to insert a stir bar magnet into each bottle. Set Bottle #1 on a stir plate and stir gently. A small vortex should be visible on the surface of the liquid.

Do not handle the stir bar with fingers. Bare fingers will add chlorine demand to the sample.

4. Open a Chlorine Dosing Solution Ampule. Using a TenSette Pipet, add 0.1 mL of the chlorine solution to Bottle #1 while stirring. Immerse the end of the pipet tip under the water to dispense the chlorine. Mixing while adding the chlorine is imperative to avoid highly localized areas of chlorine concentration.

5. Turn off the stirrer and fill the bottle until it overflows with sample. Cap and invert to mix. Record the start time and put the sample bottle in the dark or wrap with foil. Each 0.1 mL of chlorine dosing solution added will add approximately 1.0 mg/L Cl_2 to the sample.

6. Calculate the actual amount of chlorine added. See *Chlorine addition calculation* for the formula and an example.

The amounts of Dosing Solution added may be increased or decreased based on the expected organic level of the sample water and chlorine contact time.

Repeat steps 4–6

7. Repeat Steps 4–6 for bottles 2 through 6. Increase the amount of chlorine added in increments of 0.1 mL. See *Incremental addition of Cl2 dosing solution* and *Getting started*. Stagger the chlorine additions if the contact time is expected to be less than 30 minutes. This allows time to perform the chlorine analysis on each sample bottle at the specified contact time.

Method 8021
or
Method 10069

8. After the prescribed contact is completed, analyze the samples for Free Chlorine using DPD Free Chlorine Reagent. Use Method 8021 if the desired chlorine residual is below 2.0 mg/L Cl_2 or Method 10069 if residuals of greater than 2.0 mg/L Cl_2 are required. Follow the procedure supplied with the spectrophotometer or colorimeter being used.

DPD Reagent (continued)

9. Subtract the residual chlorine determined in Step 8 from the original amount of chlorine added to each bottle to determine the chlorine demand: *Cl_2 Demand = Cl_2 added concentration (mg/L) – Cl_2 residual measured concentration (mg/L)*.[1]

Report the chlorine demand according to the goals of the study.

Example: The sample dosed at 6.0 mg/L consumed 4.1 mg/L chlorine after 2 hours at 20 °C and pH 8.1.

10. Determine the chlorine requirement (chlorine dosage) needed to meet the operating goal: *Cl_2 Requirement = Cl_2 Demand + Cl_2 Residual Required*

Report the chlorine requirement according to the goals of the study.

Example: The sample required a dose of 3.0 mg/L chlorine to achieve a free chlorine residual of 1.1 mg/L chlorine after 2 hours at 20 °C and pH 8.1.

[1] Some bottles will have no residual chlorine if the chlorine demand exceeded the amount of chlorine added. Choose a bottle that has a chlorine residual to determine the demand. See *Chlorine demand results*.

Chlorine addition calculation

Use the following formula to calculate the concentration of the chlorine added in step 6.

$$\text{mg/L } Cl_2 = \frac{0.1 \text{ mL} \langle \text{volume of standard added} \rangle \times \text{ampule certificate value} \langle \text{ mg/L } Cl_2 \rangle}{125 \text{ mL}}$$

Example:

$$\text{mg/L } Cl_2 = \frac{0.1 \text{ mL} \times \text{certificate value of 1250 mg/L } Cl_2}{125 \text{ mL}}$$

mg/L Chlorine = **1.0**

Incremental reagent addition

Incremental addition of Cl2 dosing solution defines the incremental addition of a 1250 mg/L chlorine dosing solution.

Table 93 Incremental addition of Cl_2 dosing solution

Bottle #	Cl_2 dosing solution added (mL)	Increases sample concentration (in mg/L Cl_2) by
1	0.1	1.0
2	0.2	2.0
3	0.3	3.0
4	0.4	4.0
5	0.5	5.0
6	0.6	6.0

Chlorine demand results

Select the sample bottle that most closely fits the following criteria to calculate the chlorine demand.

1. Residual chlorine measured is less than the chlorine dose added (0.03* mg/L).
2. Residual chlorine measured is greater than 0.03* mg/L.
3. Chlorine dose added is most similar to the dosage range expected in the field.

Criteria 1 and 2 ensure that the chlorine residual and demand are greater than the detection limit of the DPD method used for determining the chlorine residual. If no sample portion satisfies all criteria, repeat the test and adjust the chlorine doses accordingly.

Chlorine demand test plan

Chlorine demand test plans are developed for many purposes. The purpose should be well defined and documented. This will develop the specific details of the plan, with the goal of establishing reproducible test conditions to obtain reproducible data. This data is useful in characterizing and optimizing a water treatment operation. Purposes for developing a chlorine demand test plan might be:

Characterize the water system to establish a historical data baseline.
This baseline with chlorine demand date can be used to troubleshoot water quality problems, provide background information for new employees and can provide additional support for monitoring changes in water quality. The plan would include:

- The standard elements of temperature, water pH, chlorine dose rate and chlorine contact time.
- Additional specific details to allow other analysts to reproduce the plan.

Characterize the chlorine demand of an influent raw water source.
Data obtained to establish chlorine demand data for use in understanding the effects of water source changes, blending operations and seasonal weather variations. The plan would include:

- Source water description, sample location, time of year, specific or unusual weather events

* The minimum detection limit for DPD Chlorine Method 8021 when calculating the concentrations by difference (1.412 x 0.02 mg/L).

- Additional complementary tests to be run, such as TOC, turbidity or UV-254, in addition to the standard temperature, pH, chlorine dose rate and contact time.

Track the reduction in chlorine demand as water moves through the treatment process. Data obtained would be used to establish a baseline for monitoring the effects of treatment changes, seasonal water temperatures and overall changes in chlorine demand. The plan would include:

- Specific sampling locations
- Treatment practices in use and flow rates

Getting started

Before starting this procedure, determine:

- The magnitude of the chlorine demand present in the water to be tested.
- Which chlorine method to use to determine chlorine residual.

First time users of this method or users evaluating a new water source should perform a screening test to determine an approximate chlorine demand level before performing a full chlorine demand test series.

1. Add 0.5 mL and 1.0 mL of Chlorine Dosing Solution to a 125-mL water sample.
2. Hold for the contact time indicated in the test plan and then analyze the chlorine residual.
3. Use the chlorine residual values to determine the specific dose requirements in the chlorine demand test plan. As a rule, use the HR DPD Chlorine Method (Method 10069) for raw water samples or where the desired chlorine residual will be greater than 2.0 mg/L chlorine and use the LR DPD Chlorine Method (Method 8021) for low chlorine demand waters, such as treated waters or samples where the desired chlorine residual is less than 2.0 mg/L chlorine.

Chlorine demand procedure modification

The chlorine demand procedure is an operationally defined procedure. The procedure is defined by the user and may be modified to meet the specific requirements of the sample or the process operation. Run chlorine demand studies under the range of conditions expected in the field. Use the basic test protocol while changing contact time, temperature, sample pH and chlorine concentrations. Total Chlorine or Monochloramine can be determined as required from the residual measured at the end of the prescribed contact time.

Use the following guidelines if modifications are made:

- Make smaller chlorine concentration additions by using a larger sample size. A 237-mL bottle (contains 250 mL when filled to overflowing) is available for low chlorine demand applications. Each 0.1 mL of chlorine dosing solution added will increase the chlorine concentration by approximately 0.5 mg/L. Substitute 250 mL for 125 mL in the above equation. A lower concentration Chlorine Standard Solution, 50–75 mg/L as Cl_2 is also available for testing low chlorine demand waters.
- High chlorine demand waters require larger additions of chlorine. Use 0.2 mL, 0.4 mL, 0.6 mL, etc., to spike the bottles in steps 4 and 7 in the procedure.
- Wrap sample bottles made of clear colorless glass in foil to protect from light or kept in the dark during the contact time.
- Sample pH can be modified or standardized by adding a fixed amount of a pH buffer solution to each bottle. Prepare a reagent blank bottle using organic free water. Add the same amount of buffer to this blank, add a known amount of chlorine and carry the blank through the procedure. Add only enough buffer to give the desired pH. This will check the chlorine demand (if any) that was added by the buffer. Subtract the chlorine demand of the blank from the sample chlorine demand values.

- Chlorine demand tests that have an extended contact time will require temperature control if the sample temperature is significantly different from the analysis environment. Use a refrigerator, water bath or incubator as required. It is important to control and document these variables in order to be able to duplicate the chlorine demand procedure on future samples.

Treatment of analysis labware

Glassware used in this test must be chlorine demand-free. Treat all glassware with a dilute solution of chlorine bleach prepared by adding 0.5 mL of commercial bleach to 1 liter of water. Alternatively, the sample bottles may be treated by adding 2.0 mL of the Chlorine Dosing Solution to each 125-mL bottle and filling to overflowing with deionized water. Soak glassware in this solution for at least one hour. After soaking, rinse the glassware with copious amounts of chlorine demand-free water before filling with sample.

Sampling and storage

Most reliable results are obtained on fresh low solid samples that are analyzed immediately. Samples may be stored up to 24 hours at 4 °C. Warm the samples to the required temperature before running the chlorine demand test.

Summary of method

The chlorine demand of a water sample is defined as the difference between the concentration of chlorine added to the sample and the concentration of the chlorine residual remaining at the end of a predetermined contact time. The chlorine demand is a function of chlorine concentration, sample temperature, contact time and sample pH.

The chlorine requirement is the amount of chlorine required to achieve a predetermined chlorine residual at a prescribed contact time, pH and temperature.

Chlorine demand is caused by a complex set of reactions. Chlorine reacts with dissolved or suspended organic materials in the water to form stable chlorinated organic compounds such as trihalomethanes, haloacetic acids or other chlorinated organic compounds. Some of these compounds (trihalomethanes) are referred to as disinfection by-product (DBPs) and are regulated under the Disinfection/Disinfection By-Products Rule; other chlorinated organics contribute to taste and odor problems. As a general rule, the lower the chlorine demand the lower the amounts of DBPs formed and less taste and odor problems occur. Chlorine also is reduced by inorganic reductants present such as ferrous, manganous, nitrite, sulfide and sulfite ions. Ammonia present in the water also consumes chlorine to form chloramines.

Chlorine demand is significantly impacted by the physical and chemical characteristics of the water sample. Chlorine demand studies ran at 10 °C will be considerably different than studies ran at 20 °C. It is imperative that the sample temperature, pH and chlorine dose be accurately measured and recorded. It is difficult to extrapolate chlorine demand data from one water source to another. Demand studies need to be performed directly on the water source of interest. This provides the information required to establish chlorine demand constants, to provide usable historical data and to provide the test requirements for making repeatable and meaningful chlorine demand measurements.

Consumables and replacement items

Required reagents

Description	Catalog number
DPD Free Chlorine Reagent Powder Pillows, 10 mL	2105569
OR	—
DPD Free Chlorine Reagent Powder Pillows, 25 mL	1407099
Chlorine Dosing Solution Ampules, 1190–1310 mg/L as Cl_2, 10-mL ampules, 16/pkg	2504810

Required Apparatus

Description	Catalog number
Bottles, Amber Glass, 118-mL 6/pkg	714424
Caps, Black, PP Teflon liner, 12/pkg	2401812

Optional reagents and apparatus

Description	Catalog number
Ampule Breaker, for Voluette Ampules	2196800
Bottles, Amber Glass, 237 mL, 6/pkg	714441
Bottles, Amber Glass, 1000 mL, 6/pkg	714463
Buffer Powder Pillows, pH 6.86, 15/pkg	1409895
Buffer Powder Pillows, pH 8.00, 15/pkg	1407995
Buffer Powder Pillows, pH 8.3, 15/pkg	89868
Buffer Solution, pH 7.0, demand-free, 500 mL	2155353
Caps, for 714441 Bottles, 6/pkg	2166706
Caps, for 714463 Bottles, 6/pkg	2371026
Chlorine Standard Solution Ampules, 10 mL, 50–75 mg/L as Cl_2, 16/pkg	1426810
DPD Free Chlorine AccuVacs, 25/pkg	2502025
DPD Free Chlorine SwifTest Dispenser with reagents	2802300
DPD Total Chlorine Reagent Powder Pillows, 25 mL	1406499
DPD Total Chlorine Reagent PP, 10 mL	2105969
DPD Total Chlorine SwifTest Dispenser with reagents	2802400
Graduated Cylinder, plastic, 100 mL	108142
Incubator, Model 205, 110 V, 0 to 40 °C	2616200
Labels, PolyPaper, 1.5 x 3 inches, 120/pkg	2091502
Monochlor F Reagent Powder Pillows, 10 mL	2802299

Optional reagents and apparatus (continued)

Description	Catalog number
Sens***ion*** 2 Portable pH/ISE Meter, with electrode	5172510
Sodium Hydroxide Standard Solution, 0.100N, 500 mL	19153
Standard Methods Handbook	2270800
Stir Bar, Teflon-coated, 2.22 cm x 0.48 cm	4531500
Stir Plate, 120 V, 4.25 x 4.25 inches	2881200
Sulfuric Acid Std. Solution, 0.100 N, 500 mL	20253
TenSette Pipet, 0.1 - 1.0 mL	1970001
Tips for TenSette Pipet, 0.1-1.0 mL, 50/pkg	2185696
Thermometer, Double Scale, –20 to 110 °C (0–230 °F)	2095911
Tweezers	1428200
Water, Organic-Free 500 mL	2641549

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Edition 7

Chlorine, Free

DOC316.53.01026

DPD Rapid Liquid Method[1] **Method 10059**

(0.02 to 2.00 mg/L) **Pour-Thru™ Cell**

Scope and Application: For treated water.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 94 Instrument-specific information

Instrument	Pour-thru Kit	Cell orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path in line with the arrow on the adapter	LZV585 (B)

Before starting the test:

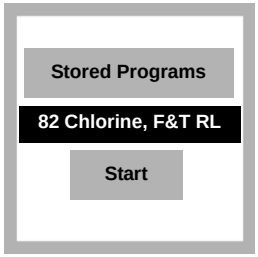
Analyze samples immediately. Do not preserve for later analysis.
Refer to the instrument User Manual for Pour-Thru cell and module assembly and installation.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
The indicator reagent must be prepared in advance. See *Reagent preparation*.
Make sure the pour-thru cell is completely seated in the sample cell compartment.

Collect the following items:

Description	Quantity
DPD Indicator Powder	varies
Free Chlorine Indicator Solution	1 mL
Free Chlorine Buffer Solution	1 mL
Cylinder, glass, mixing, 100-mL	1
Dispenser, Adjustable Volume	2
Pour-Thru Cell Module and Cell (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

Free Chlorine, rapid liquid method

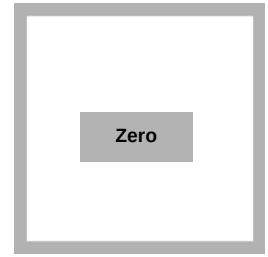


1. Select the test.

(See *Instrument-specific information*).

2. Install the Pour-Thru Cell module and cell. See *Instrument-specific information* for adapter and cell orientation.

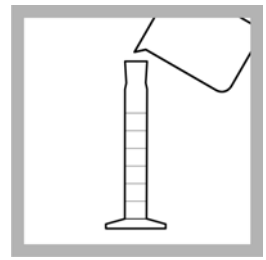
3. Pour approximately 50 mL of sample into the Pour-Thru Cell.



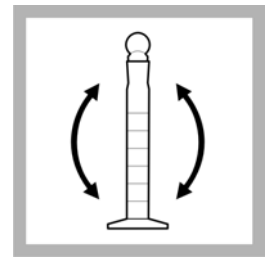
4. When the flow stops, press **ZERO**. The display will show: 0.00 mg/L Cl_2.

5. Add 1.0 mL of Free Chlorine Buffer Solution to a clean, dry 100-mL glass mixing cylinder using the Dispenser.

6. Add 1.0 mL of prepared Free Chlorine Indicator Solution to the same mixing cylinder using the dispenser. Swirl to mix the reagents. Proceed to step 7 immediately.



7. Carefully fill the mixing cylinder to the 80-mL mark with sample.



8. Stopper the cylinder and gently invert it twice to mix. Proceed to step 8 immediately.

Free Chlorine, rapid liquid method (continued)

9. Fill the funnel of the Pour-Thru Cell with the reacted sample from the mixing cylinder.

(It is not necessary to pour the entire sample into the Pour-Thru Cell; approximately half of the sample may be discarded.)

10. After the flow stops, press **READ**. Results will appear in mg/L Cl_2.

11. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use.

Reagent preparation

The Free Chlorine Indicator Solution must be prepared before use. Using a powder funnel, add the contents of one 24 g bottle of DPD Powder* to one 473-mL bottle of Free Chlorine Indicator Solution*. Invert several times and swirl until the powder is completely dissolved. A pale pink color may develop, but should not affect results.

This solution will give accurate results for at least one month after mixing when stored at 20–25 °C (68–77 °C). Write the date of preparation on the Indicator Solution Bottle. Discard any remaining solution after one month. Use of this reagent after one month may result in high reagent blanks and low values at high concentration. Do not combine fresh reagent with previously mixed reagent.

Interferences

Table 95 Interfering substances

Interfering substance	Interference level
Alkalinity	Greater than 400 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels.
Hardness	Levels below 1000 mg/L as $CaCO_3$ will not interfere.
Iodine, I_2	Interferes at all levels.

* See *Required reagents*.

Table 95 Interfering substances (continued)

Interfering substance	Interference level
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid[1]. **2.** Add 9 drops Potassium Iodide (30 g/L)[1] to an 80-mL sample. **3.** Mix and wait 1 minute. **4.** Add 9 drops Sodium Arsenite[1, 2] (5 g/L) and mix. **5.** Analyze the treated sample as described in the procedure above. **6.** Subtract the result of this test from the original analysis to obtain the correct concentration.
Monochloramine (NH_2Cl)	Samples containing monochloramine will cause a gradual drift to higher chlorine readings. When read within one minute of reagent addition, 3.0 mg/L monochloramine will cause an increase of less than 0.1 mg/L in the free chlorine reading.
Ozone	Interferes at all levels.

[1] See *Optional standards and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). Refer to the current MSDS for safe handling and disposal instructions.

Sampling and storage

Samples must be analyzed immediately and cannot be preserved for later analysis.

A common testing error is introduced if the analyst does not obtain a representative sample. If sampling from a tap, let the water flow for at least five minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Avoid plastic containers since these may have a chlorine demand.

Pre-treat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized water.

If sample containers are rinsed thoroughly with deionized water after use, only occasional pretreatment is necessary. A pre-treated glass BOD bottle with a ground-glass stopper makes an ideal sample container for chlorine collection.

Treating analysis labware

Glassware used in this test must be chlorine demand-free. Fill the 100-mL mixing cylinder and sample container with a dilute solution of chlorine bleach prepared by adding 1 mL of commercial bleach to 1 liter of water. Soak in this solution at least one hour. After soaking, rinse thoroughly with deionized water and allow to dry before use. If the mixing cylinder is thoroughly rinsed with deionized water and allowed to dry after each use, only occasional pretreatment is necessary. Do not use the same mixing cylinder for Free and Total Chlorine analysis.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes. Rinse several times with deionized water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N Sulfuric Acid followed by several rinsings with deionized water.

Accuracy check

Required for accuracy check:

- Chlorine Voluette® Ampule Standard Solution, 50 to 75-mg/L Cl_2
- TenSette Pipet and tips

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Chlorine Voluette® Ampule Standard Solution, 50 to 75-mg/L Cl_2.
5. Prepare three sample spikes. Use the TenSette® Pipet to add 0.3, 0.6 and 0.9 mL of standard to three 80-mL samples, respectively. Swirl gently to mix.
6. Analyze each sample spike as described in the procedure above, starting with the 0.3 mL sample spike. Accept each standard additions value. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
82	1.18 mg/L Cl_2	1.17–1.19 mg/L Cl_2	0.02 mg/L Cl_2

Summary of method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a pink color which is proportional to the chlorine concentration. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Free Chlorine Reagent Set, includes:	—	—	2556900
DPD Indicator Powder	varies	24 g	2297255
Free Chlorine Indicator Solution	1 mL	473 mL	2314011
Free Chlorine Buffer Solution	1 mL	473 mL	2314111

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, mixing graduated, 100-mL, glass	1	each	2636342
Dispenser, adjustable-volume, 1.0 – 5.0 mL	2	each	2563137
Powder Funnel	1	each	2264467

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, Voluette® Ampule, 50-75 mg/L, 10-mL	16/pkg	1426810
OR		
Chlorine Standard Solution, Pour-Rite® Ampule, 50-75 mg/L, 2-mL	20/pkg	1426820
Voluette Ampule breaker, 10 mL	each	2196800
PourRite Ampule breaker 2-mL	each	2484600
Water, deionized	4 L	27256

Optional standards and apparatus

Description	Unit	Catalog number
Pipet, TenSette® 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
pH Paper, 0 - 14 pH range	100/pkg	2601300
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sulfuric Acid, 1 N	100 mL	127032
Sulfuric Acid, 5.25 N	100 mL	244953

[1] Other sizes are available

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Edition 7

Chlorine, Free

DOC316.53.01024

DPD Method[1] **Method 10102**

(0.09 to 5.00 mg/L) **Test ‘N Tube™ Vials**

Scope and Application: For testing higher levels of free chlorine (hypochlorous acid and hypochlorite ion) in drinking water, cooling water and industrial process water.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 96 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

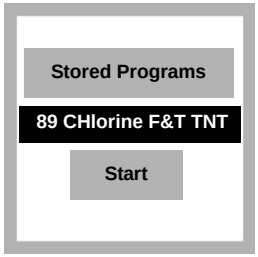
DR 3900, DR 3800, DR 2700 and DR 2800: Install the light shield in Cell Compartment #2 before performing this test.
Analyze samples immediately. Do not preserve samples for later analysis.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
After adding sample to the Test ‘N Tube™, a pink color will develop if free chlorine is present.

Collect the following items:

Description	Quantity
Light Shield (see *Instrument-specific information*)	1
Test ‘N Tube™ DPD Free Chlorine Reagent	1
Wipes, disposable	varies

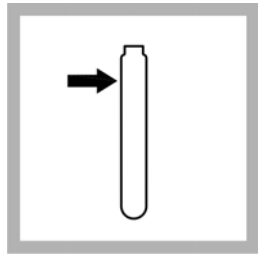
See *Consumables and replacement items* for reorder information.

DPD method for Test ‘N Tube vials



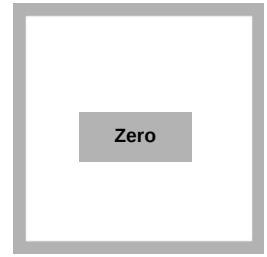
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Blank Preparation: Fill an empty Test ‘N Tube vial to the top of the label with sample.

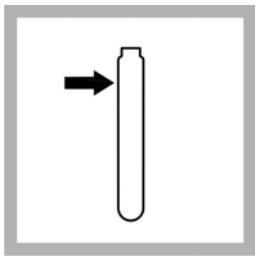
3. Wipe the blank and insert it into the 16 mm cell holder.



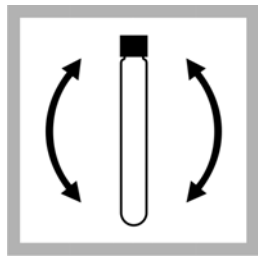
4. ZERO the instrument.

The display will show:

0.00 mg/L Cl_2

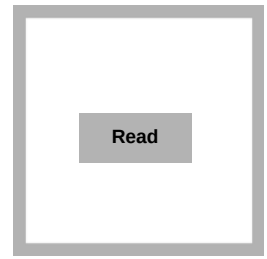


5. Remove the cap from a Free Chlorine DPD Test ‘N Tube. Add 10 mL of sample to the tube. Fill the vial to the top of the label.



6. Prepared Sample: Cap and invert slowly at least 10 times to dissolve the powder. Invert by turning the vial upside down, then returning it to an upright position. Ten inversions should take at least 30 seconds for complete recovery.

7. Immediately wipe the sample and insert it in the 16 mm cell holder.



8. READ the results in mg/L Cl_2.

Interferences

Table 97 Interfering substances and levels

Interfering substance	Interference levels and treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N sodium hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere

Table 97 Interfering substances and levels (continued)

<table>
<tr><th>Interfering substance</th><th>Interference levels and treatments</th></tr>
<tr><td>Hardness</td><td>No effect at less than 1000 mg/L as $CaCO_3$</td></tr>
<tr><td>Iodine, I_2</td><td>Interferes at all levels</td></tr>
<tr><td>Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})</td><td>Adjust sample pH to 6–7.
Add 3 drops potassium iodide[1] (30-g/L) to a 25-mL sample.
Mix and wait 1 minute.
Add 3 drops sodium arsenite[1,2] (5-g/L) and mix.
Analyze 10 mL of the treated sample as described in the procedure.
Subtract the result from this test from the original analysis to obtain the correct chlorine concentration in the sample.</td></tr>
<tr><td>Monochloramine</td><td>For conventional free chlorine disinfection (beyond the breakpoint), typical monochloramine concentrations are very low. If monochloramine is present in the sample, its interference in the free chlorine test depends on the sample temperature, relative amount of monochloramine to free chlorine and the time required to do the analysis. Typical interference levels of monochloramine as mg/L Cl_2 in the free chlorine test are listed below (1 minute test time).
<table>
<tr><th rowspan="2">NH_2Cl (as Cl_2)</th><th colspan="4">Sample Temp. °C (°F)</th></tr>
<tr><th>5 (41)</th><th>10 (50)</th><th>20 (68)</th><th>30 (83)</th></tr>
<tr><td>1.2 mg/L</td><td>+0.15</td><td>0.19</td><td>0.30</td><td>0.29</td></tr>
<tr><td>2.5 mg/L</td><td>+0.35</td><td>0.38</td><td>0.55</td><td>0.61</td></tr>
<tr><td>3.5 mg/L</td><td>+0.38</td><td>0.56</td><td>0.69</td><td>0.73</td></tr>
</table>
Note: Determine Monochloramine levels using Hach method 10200.</td></tr>
<tr><td>Ozone, O_3</td><td>Interferes at all levels</td></tr>
<tr><td>Peroxides</td><td>May interfere</td></tr>
<tr><td>Extreme sample pH or highly buffered samples</td><td>Adjust to pH 6–7 using acid (Sulfuric Acid[1], 1.000 N) or base (Sodium Hydroxide[1], 1.00 N).</td></tr>
</table>

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). Refer to reagent MSDS for disposal instructions.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Accuracy check

Required for accuracy check:

- Chlorine PourRite® Ampule Standard*, 50–75 mg/L Cl_2
- TenSette Pipet 0.1 – 1.0 mL and tips
- Ampule Breaker

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify that the units displayed are in mg/L.
2. Select Options>More>Standard Additions from the instrument menu.
3. A keypad will appear. Enter the average chlorine concentration shown on the label of the standard solution. Press **OK**. A summary of the Standard Additions procedure will appear. Press **OK**.
4. Open a Chlorine PourRite® Ampule Standard*, 50–75 mg/L Cl_2.
5. Use the TenSette® Pipet to add 0.1 mL to a 10-mL sample. Mix thoroughly.
6. Analyze the standard addition sample as described in the procedure above. Accept the standard additions reading by pressing **READ**. The addition should reflect approximately 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
89	2.68 mg/L Cl_2	2.63–2.73 mg/L Cl_2	0.03 mg/L Cl_2

Summary of method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a pink color which is proportional to the chlorine concentration. Test results are measured at 530 nm.

* See *Optional reagents and apparatus*.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Test ‘N Tube™ DPD Free Chlorine Reagent	1	50/pkg	2105545

Recommended standards and apparatus

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
Voluette Ampule breaker 10 mL	each	2196800
PourRite Ampule breaker 2-mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Wipes, disposable®	280/pkg	2097000
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Sulfuric Acid, 1.000 N	100 mL	127032
Sodium Hydroxide, 1.00 N	100 mL	104532
pH Paper, 0 - 14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, -10 to 225°C	each	2635700
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Test Tube Rack	each	1864100
Potassium Iodide, 30-g/L	100mL	34332
Sodium Arsenite, 5-g/L	100mL	104732

[1] Other sizes are available

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Edition 7

Chlorine, Free

DOC316.53.01023

USEPA DPD Method[1] **Method 8021**

(0.02 to 2.00 mg/L) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For testing free chlorine (hypochlorous acid and hypochlorite ion) in water, treated waters, estuary and seawater. USEPA accepted for reporting for drinking water analyses.[2]

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

[2] Procedure is equivalent to USEPA and Standard Method 4500-Cl G for drinking water.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 98 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

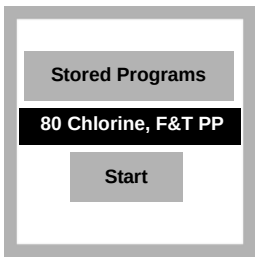
If the test over-ranges, dilute the sample with a known volume of high quality, chlorine demand-free water and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. Alternatively, samples with high chlorine concentrations may be analyzed directly without dilution by using Method 10069, Chlorine, Free HR, or Method 10245, Chlorine Free MR .
The SwifTest Dispenser for Free Chlorine can be used in place of the powder pillow in step 4.
Analyze samples immediately. Do not preserve for later analysis.
The sample cell shown is a generic representation. Refer to *Instrument-specific information* for the correct sample cell and adapter configuration.
An empty AccuVac ampule can be used as a blank in place of the sample cell in Step 2.
Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
DPD Free Chlorine Reagent Powder Pillows, 10-mL	1
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
DPD Free Chlorine Reagent AccuVac® Ampuls	1
Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

Powder pillow procedure



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Blank Preparation: Fill a sample cell with 10 mL of sample.

3. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show:

0.00 mg/L Cl_2

4. Prepared Sample: Fill a second cell with 10 mL of sample.

Add the contents of one DPD Free Chlorine Powder Pillow to the sample cell.

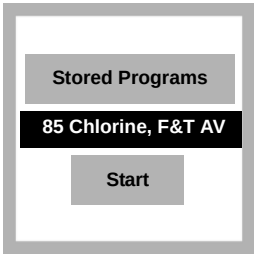
5. Swirl the sample cell for 20 seconds to mix.

A pink color will develop if chlorine is present. Proceed to step 6 immediately.

6. Within one minute of adding the reagent, insert the prepared sample into the cell holder.

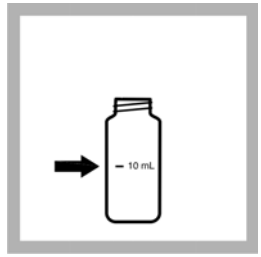
Results are in mg/L Cl_2.

AccuVac Ampuls procedure



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



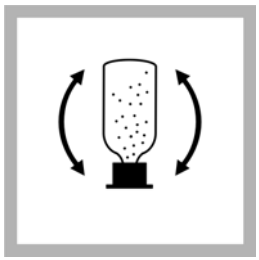
2. Blank Preparation: Fill a sample cell with 10-mL of sample.

3. Wipe the blank and insert it into the cell holder.

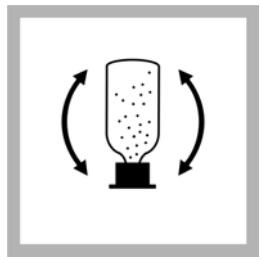
ZERO the instrument. The display will show: 0.00 mg/L Cl_2

4. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill a DPD Free Chlorine Reagent AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.



5. Quickly invert the Ampul several times to mix. Wipe off any liquid or fingerprints.



6. Within one minute after sample addition, wipe the AccuVac Ampul and insert it into the cell holder.

READ the results in mg/L Cl_2

Interferences

Table 99 Interfering substances and levels

Interfering substance	Interference levels and treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as $CaCO_3$
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30-g/L) to a 10-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops Sodium Arsenite [1] (5-g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Monochloramine	Causes a gradual drift to higher readings. When read within 1 minute after reagent addition, 3 mg/L monochloramine causes less than a 0.1 mg/L increase in the reading.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid, 1.000 N) or base (Sodium Hydroxide, 1.00 N).

[1] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See the current MSDS for proper disposal of hazardous material.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Accuracy check

Standard additions method (Sample spike)
Required for accuracy check:

- Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L
- Breaker, PourRite Ampules
- Pipet, TenSette®, 0.1–1.0 mL and tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Enter the average chlorine concentration shown on the label of the ampule container.
4. A summary of the standard additions procedure will be displayed. Press **OK** to accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row.
5. Open one Voluette ampule standard.
6. Prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.

   Note: *For AccuVac® Ampuls, add 0.4 mL, 0.8 mL and 1.2 mL of standard to three 50-mL portions of fresh sample.*

7. Follow the test procedure for each of the spiked samples using the powder pillows or AccuVac ampules, starting with the smallest sample spike. Measure each of the spiked samples in the instrument.
8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Note: *If results are not within acceptable limits (± 10%), be sure that the sample volumes and sample spikes are measured accurately. The sample volumes and sample spikes that are used should agree with the selections in the standard additions menu. If all procedures are followed correctly but the standard additions results are not within acceptable limits, the sample may contain an interference.*

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
80	1.25 mg/L Cl_2	1.23–1.27 mg/L Cl_2	0.02 mg/L Cl_2
85	1.25 mg/L Cl_2	1.21–1.29 mg/L Cl_2	0.02 mg/L Cl_2

Summary of method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a pink color, the intensity of which is proportional to the chlorine concentration. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
DPD Free Chlorine Reagent Powder Pillows, 10-mL	1	100/pkg	2105569
OR			
DPD Free Chlorine Reagent AccuVac® Ampuls	1	1	2502025

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
AccuVac Snapper	1	each	2405200
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
PourRite Ampule breaker, 2-mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Chlorine-demand Free Water	500 mL	2641549
Cylinder, mixing, 25 mL	each	2088640
Cylinder, mixing, 50 mL	each	189641
Sodium Hydroxide, 1 N	100 mL	104532
Sulfuric Acid, 1 N	100 mL	127032
Potassium Iodide, 30-g/L	100 mL	34332
Sodium Arsenite, 5-g/L	100 mL	104732
SwifTest Dispenser for Free Chlorine[1]	each	2802300
Pipet, TenSette®, Pipet, 0.1 - 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
pH Paper, 0 - 14 pH range	100/pkg	2601300
Voluette Ampule breaker, 10 mL	each	2196800
AccuVac, vials for sample blanks	25/pkg	2677925
Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
DPD Free Chlorine Reagent Powder Pillows, 10-mL	1000/pkg	2105528
DPD Free Chlorine Reagent Powder Pillows, 10-mL	300/pkg	2105503
DPD Free Chlorine Reagent, 10 mL, SwifTest Dispenser refill vial	250 tests	2105560
SpecCheck Secondary Standard Kit, Chlorine DPD, 0–2.0 mg/L Set	each	2635300

[1] Includes one vial of 2105560 for 250 tests

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Edition 7

Chlorine, Free

DOC316.53.01155

USEPA[1] Amperometric Buret Titration Method[2]

Method 8334

0.5 mg/L and above

Buret Titration

Scope and Application: For water and wastewater.

[1] USEPA accepted; 40 CFR Part 141, Section 141.74.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater* (4500 Cl^-D).

Test preparation

Before starting the test:

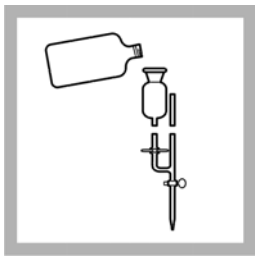
Chlorine can be lost from the sample during sample collection. Review the precautions in *Sample collection, preservation and storage* before the test is started.
Use only a 50-mm stir bar. The wrong size can cause the loss of chlorine, unstable readings and loss of method sensitivity, especially when measuring low level chlorine concentrations.
For added convenience when stirring, use the TitraStir® apparatus.
When a new probe is placed in service or when the probe has not been used recently, prepare it according to the Probe Stabilization instructions in the Amperometric Titrator Instruction Manual.

Collect the following items:

Description	Quantity
Phenylarsine Oxide Solution, 0.00564 N	1 bottle
Phosphate Buffer Solution, pH 7	1 mL
Amperometric Buret Titrator System	1
Beaker, 250-mL	1
Graduated cylinder, 250-mL	1

See *Consumables and replacement items* for reorder information.

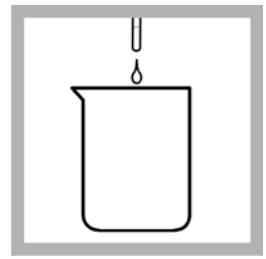
Buret titration



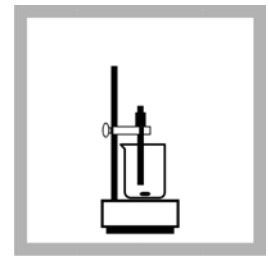
1. Fill the 5-mL automatic buret to the zero mark with 0.00564 N Phenylarsine Oxide (PAO) Solution.

2. Put a 50-mm stir bar into a 250-mL beaker.

Use a graduated cylinder to measure 200 mL of sample. Add the sample to the beaker.

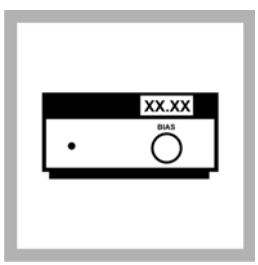


3. If the pH is less than 6 or greater than 7.5, add 1.0 mL of pH 7 Phosphate Buffer Solution to make the prepared sample.



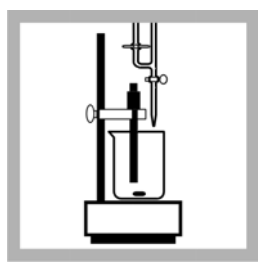
4. Place the beaker of prepared sample on the TitraStir titration stand and turn on the stirring motor. Put the tip of the probe fully into the prepared sample. The platinum wires must be submerged.

If a stir plate other than the TitraStir® is used, set the speed for moderate mixing. Do not adjust the speed after this point.

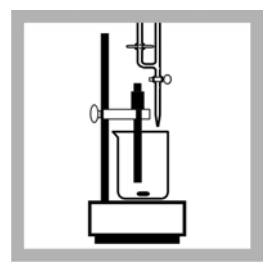


5. Turn the BIAS control knob to adjust the value on the display to approximately 1.00.

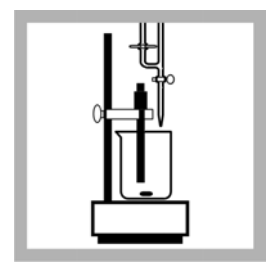
The BIAS adjustment controls the slope of the titration curve. The actual value is not important. Only the relative value as the titration continues is important. A precise adjustment is not necessary.



6. Dispense the titrant into the beaker in small increments while monitoring the values on the Amperometric Titrator. The values will decrease.

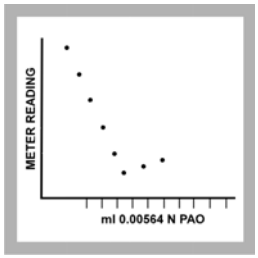


7. Continue dispensing slowly. Near the end point of the titration, write down the value on the display and the corresponding total volume of titrant that was added. Read the volume to the nearest 0.01 mL. Add a small amount of titrant and wait several seconds for a stable value. Write down the value.

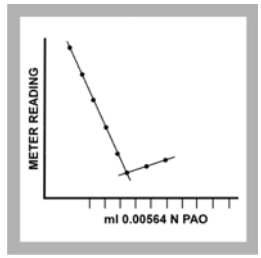


8. Continue the titration by recording at least three points on the downward sloping curve and at least three points after the end point has been reached. The value on the display will not change significantly after the end point.

Buret titration (continued)



9. Make a graph of the titration. Plot the values from the amperometric titrator on the vertical axis and the corresponding volume of titrant on the horizontal axis.



10. Draw the two best intersecting lines through the points as shown above. Find the volume of titrant to the nearest 0.01 mL at the intersection of the two lines. This is the mL titrant end point. This volume is equivalent to the free chlorine concentration in mg/L.

mL titrant =
mg/L free chlorine as Cl_2

Interferences

Refer to the Amperometric Titrator Instruction Manual for a discussion of sources of errors and interferences using the amperometric methods.

Sample collection, preservation and storage

Start the chlorine analysis immediately after the samples are collected. Chlorine is a strong oxidizing agent and is not stable in natural waters. Chlorine reacts quickly with various inorganic compounds and slowly oxidizes organic compounds. Many factors such as sample composition, sunlight, pH, temperature and salinity can cause the decomposition of chlorine in water.

Do not use plastic containers because plastic can react with and consume chlorine. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of demineralized water) for at least 1 hour. Rinse thoroughly with demineralized or distilled water. If sample containers are rinsed thoroughly with demineralized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample containers for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample containers for free and total chlorine determinations.

A common error in testing for chlorine is introduced when a representative sample is not obtained. If sampling from a tap, let the water flow for at least 5 minutes before sample collection. Let the sample container overflow with the sample several times, then cap the container so that there is no headspace (air) above the sample. Start the chlorine analysis immediately.

Summary of method

Free chlorine is measured by a titration at pH 7 with PAO solution to the amperometric end point. The amperometric titration method has greater sensitivity and accuracy when compared to colorimetric methods. Refer to the Amperometric Titrator Instruction Manual for more information.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Phenylarsine Oxide Solution, 0.00564 N	varies	1 L	199953
Phosphate Buffer Solution, pH 7	1 mL	100 mL MDB	2155332

Required apparatus

Description	Unit	Catalog number
Amperometric Buret Titrator System, 115 VAC	each	1930010
Beaker, 250-mL	each	50046H
Graduated Cylinder, 250-mL	each	50846
Stir bar, 50 mm	each	2095355
TitraStir® apparatus, 115 VAC	each	1940000
TitraStir apparatus, 230 VAC	each	1940010
pH Paper, 0–14 pH range	100/pkg)	2601300

Optional reagents and apparatus

Description	Unit	Catalog number
Chlorine Standard Solution, 10-mL Voluette® Ampules, 50–75 mg/L	16/pkg	1426810
Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 2-mL PourRite Ampule, 25–30 mg/L	20/pkg	2630020
Voluette Ampule breaker 10-mL	each	2196800
PourRite Ampule breaker 2-mL	each	2484600
Water, deionized	500 mL	27249

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Edition 7

Chlorine, Free

DOC316.53.01025

DPD Method[1] **Method 10069**

HR (0.1 to 10.0 mg/L as Cl_2) **Powder Pillows**

Scope and Application: For testing higher levels of free chlorine (hypochlorous acid and hypochlorite ion) in drinking water, cooling water and industrial process waters

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 100 Instrument-specific information

Instrument	Sample cell	Cell orientation	Adapter
DR 6000	4864302	Orientation key toward user	A23618
DR 5000	4864302	Orientation key toward user	A23618
DR 3900	4864302	Orientation key away from user	A23618
DR 3800, DR 2800, DR 2700	5940506	1-cm (flat) path in line with the indicator arrow on the adapter	LZV585 (B)

Before starting the test:

Analyze samples immediately. Do not preserve for later analysis.
If the chlorine concentration is less than 2 mg/L, use Method 8021, program number 80.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

Collect the following items:

Description	Quantity
DPD Free Chlorine Reagent Powder Pillows	1
Sample Cell (see *Instrument-specific information*)	1

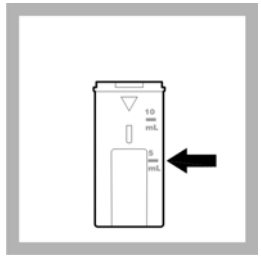
See *Consumables and replacement items* for reorder information.

Multi-path Cell

1. Select the test.

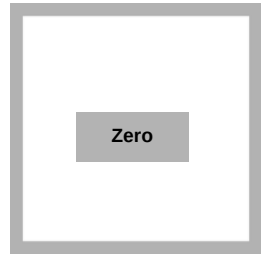
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill the sample cell to the 5-mL line with sample.

3. Wipe the cell and insert it into the cell holder.

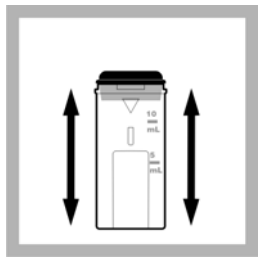


4. **ZERO** the instrument.

The display will show:

0.0 mg/L Cl_2

5. Remove the cell and add the contents of one DPD Free Chlorine powder pillow for 25-mL samples to the sample.



6. Cap and shake the cell about 20 seconds to dissolve reagent.

A pink color will develop if chlorine is present.

7. Insert the prepared sample into the cell holder.

READ the results in mg/L Cl_2.

Interferences

Table 101 Interfering substances and levels

Interfering substance	Interference level
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N Sodium Hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Iodine, I_2	Interferes at all levels

Table 101 Interfering substances and levels (continued)

<table>
<tr><th>Interfering substance</th><th>Interference level</th></tr>
<tr><td>Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})</td><td>1. Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid[1].
2. Add 2 drops Potassium Iodide[1] (30 g/L) to a 5-mL sample.
3. Mix and wait 1 minute.
4. Add 2 drops Sodium Arsenite[1, 2] (5 g/L) and mix.
5. Analyze the treated sample as described in the procedure above.
6. Subtract the result of this test from the original analysis to obtain the correct concentration.</td></tr>
<tr><td>Monochloramine (NH_2Cl)</td><td>For conventional free chlorine disinfection (beyond the “breakpoint”), typical monochloramine concentrations are very low. If monochloramine is present in the sample, its interference in the free chlorine test is dependent on sample temperature, relative concentration of monochloramine to free chlorine and the time required to perform the analysis.
Typical interference levels of NH_2Cl (1 minute test time, interference as mg/L Cl_2):
<table>
<tr><th rowspan="2">NH_2Cl Level (as Cl_2)</th><th colspan="4">Sample Temperature °C (° F)</th></tr>
<tr><th>5 (41)</th><th>10 (50)</th><th>20 (68)</th><th>30 (86)</th></tr>
<tr><td>1.2 mg/L</td><td>+0.15</td><td>0.19</td><td>0.30</td><td>0.29</td></tr>
<tr><td>2.5 mg/L</td><td>+0.35</td><td>0.38</td><td>0.55</td><td>0.61</td></tr>
<tr><td>3.5 mg/L</td><td>+0.38</td><td>0.56</td><td>0.69</td><td>0.73</td></tr>
<tr><td>5.0 mg/L</td><td>+0.68</td><td>0.75</td><td>0.93</td><td>1.05</td></tr>
</table>
Note: Determine Monochloramine levels using Hach method 10200.</td></tr>
<tr><td>Ozone</td><td>Interferes at all levels</td></tr>
<tr><td>Peroxides</td><td>May interfere</td></tr>
<tr><td>Extreme sample pH or highly buffered samples</td><td>Adjust to pH 6–7 using acid (Sulfuric Acid[1]) or base (Sodium Hydroxide[1]).</td></tr>
</table>

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). Refer to the current MSDS for safe handling and disposal instructions.

Sampling and storage

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and reacts rapidly with various compounds. Many factors such as sunlight, pH, temperature and sample composition will influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over to the free chlorine test, monochloramine could interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 5-mL mark. Proceed with the chlorine test immediately.

Accuracy check

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Chlorine PourRite® Ampule Standard, 50–75 mg/L.
5. Prepare three sample spikes. Fill three mixing cylinders* with 5-mL of sample. Using the TenSette® Pipet*, add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
88	5.4 mg/L Cl_2	5.3–5.5 mg/L Cl_2	0.04 mg/L Cl_2

Summary of method

The range of analysis using the DPD method for free chlorine can be extended by adding more indicator in proportion to sample volume. Thus, a larger fill powder pillow of DPD Free Chlorine Reagent is added to a 5-mL sample portion.

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N, N-diethyl-p-phenylenediamine) indicator to form a pink color which is proportional to the chlorine concentration. Test results are measured at 530 nm.

* See *Optional reagents and apparatus*.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
DPD Free Chlorine Reagent Powder Pillows for 25-mL samples	1	100/pkg	1407099

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL PourRite® Ampules, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 2-mL PourRite® Ampules, 25–30 mg/L	20/pkg	2630020
Chlorine Standard Solution, 10-mL Voluette® Ampules, 50–75 mg/L	16/pkg	1426810
Spec √ Gel Secondary Standard Kit, Chlorine DPD, 0–10 mg/L	4/pkg	2893300
Voluette Ampule breaker, 10 mL	each	2196800
PourRite Ampule breaker 2-mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25 mL tall form	each	2088640
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
pH Paper, 0–14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
DPD Free Chlorine Reagent Powder Pillows, 10-mL	1000/pkg	2105528
DPD Free Chlorine Reagent Powder Pillows, 10-mL	300/pkg	2105503
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sodium Hydroxide, 1N	100 mL	104532
Sulfuric Acid, 1N	100 mL	127032

[1] Other sizes are available

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Edition 7

Chlorine, Free

DOC316.53.01256

Indophenol[1] **Method 10241**

(0.04 to 4.50 mg/L Cl_2) **Powder Pillows**

Scope and Application: For determining residual free chlorine levels in the presence of manganese, chloramines and other oxidants which interfere with DPD colorimetric, DPD titrimetric, and amperometric methods for free chlorine. For use in potable water, chlorinated drinking water, swimming pool water and treated wastewater effluent.

[1] *Patent pending.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table shows requirements that can vary between instruments. To use this table, select an instrument, then read across to find the corresponding information required to perform this test.

Table 102 Instrument-specific information

Instrument	Sample cell	Cell orientation	Adapter
DR 6000	4864302	Orientation key toward user	A23618
DR 5000	4864302	Orientation key toward user	—
DR 3900	4864302	Orientation key inserted in adapter slot	LZV846 (A)
DR 3800, DR 2800, DR 2700	5940506	1-cm (flat) path aligned with arrow on adapter	LZV585 (B)

Before starting the test:

Put the protective cover or lid over the cell compartment during measurements.
This method uses Program 66, Monochloramine LR.
The sample and reagent from one analysis can contaminate other analyses and interfere with the test results. Be sure to rinse the cells and caps several times with deionized water or with the sample water to be tested before each test.
Do not switch the sample cell caps between the Blank and Sample during the analysis.
Tap sample cells lightly on a hard surface or slowly invert the cells to remove air bubbles from the cell walls.
Be sure to keep the cap on the sample cells when not used to avoid ammonia contamination.
Dispose of reacted solutions according to local, state and federal regulations. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.

Collect the following items:

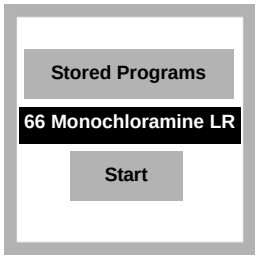
Description	Quantity
Freechlor F Reagent Solution	5 drops
Monochlor F Reagent Pillows	2

Collect the following items: (continued)

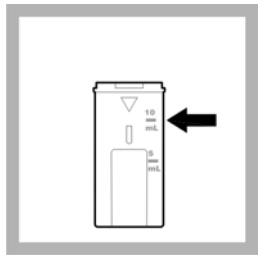
Description	Quantity
Sample cell (refer to *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

Indophenol Method



1. Select the test. Insert the correct adapter (refer to *Instrument-specific information*).



2. Fill 2 sample cells to the 10-mL line with sample. Mark one cell as the **Blank**. Mark the other cell as the **Sample**.

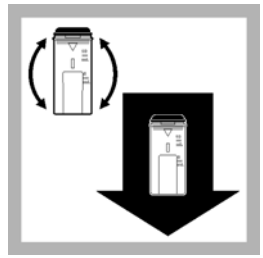
3. Add 5 drops of the Freechlor F Reagent to the **Sample** cell.

4. Cap and invert the cell to mix.

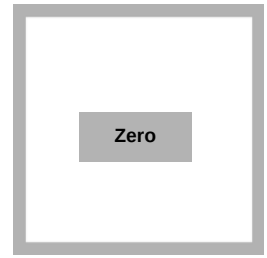
5. Add the contents of one Monochlor F Reagent Powder Pillow to the **Blank** cell and to the **Sample** cell. Cap and shake both cells 20 seconds to dissolve.

6. Press **TIMER**>**OK**. A 5-minute reaction will start.

If the sample temperature is less than 18 °C (64 °F), refer to Table 103 for the correct reaction time.



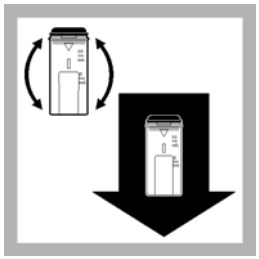
7. When the timer stops, invert the **Blank** cell to mix. Insert the cell into the cell holder.



8. **ZERO** the instrument.

The display will show:
0.00 mg/L Cl_2

Indophenol Method (continued)



9. Invert the **Sample** cell to mix. Insert the cell into the cell holder. **READ** the results in mg/L Cl_2.

Color reaction time

Use Table 103 to find the reaction time when the sample temperature is less than 18 °C (64 °F). The color is stable for 30 minutes after the reaction time is reached. If a temperature measurement is not available, read the sample after the color is stable.

Table 103 Color development based on sample temperature

Sample Temperature		Reaction Time (minutes)
°C	°F	
5	41	10
7	45	9
9	47	8
10	50	8
12	54	7
14	57	7
16	61	6
18	64	4
20	68	3
23	73	2.5
25	77	2
greater than 25	greater than 77	2

Interferences

The substances listed in Table 104 have been tested for interference and do not interfere at or below the indicated levels. Refer to Table 105 for substances that do interfere with the test.

Table 104 Non-interfering substances

Interfering substance	Interference level
Alanine	1 mg/L N
Aluminum	10 mg/L Al^{3+}
Bromide	100 mg/L Br^-

Table 104 Non-interfering substances (continued)

Interfering substance	Interference level
Bromine	15 mg/L Br_2
Calcium	1000 mg/L as $CaCO_3$
Chloride	18,000 mg/L Cl^-
Chlorine Dioxide	5 mg/L ClO_2
Chromium (III)	5 mg/L Cr^{3+}
Copper	10 mg/L Cu
Cyanide	10 mg/L CN^-
Dichloramine	10 mg/L as Cl_2
Fluoride	5 mg/L F^-
Glycine	1 mg/L N
Iodine	4 mg/L I_2
Iron (II)	10 mg/L Fe^{2+}
Iron (III)	10 mg/L Fe^{3+}
Lead	10 mg/L Pb
Manganese (7+)	3 mg/L MnO_4^-
Nitrate	100 mg/L NO_3^-–N
Nitrite	50 mg/L NO_2^-–N
Oxone®[1] (potassium peroxomonopersulfate)	30 mg/L
Phosphate	100 mg/L PO_4^{3-}
Silica	100 mg/L SiO_2
Sulfate	2600 ppm SO_4^{2-}
Tyrosine	1 mg/L N
Urea	10 mg/L N
Zinc	5 mg/L Zn^{2+}

[1] Oxone is a registered trademark of E.I. du Pont de Nemours & Co., Inc.

Table 105 Interfering substances

Interfering substance	Interference level
Ozone[1]	> 1 mg/L O_3
Sulfide[1]	> 0.5 mg/L S^{2-}

[1] This compound does not usually exist with free chlorine.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence the decomposition of free chlorine in water.
- Avoid plastic containers because they can have a large chlorine demand.

- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Testing applications

Finished chlorinated drinking waters and distributions systems
Finished waters contain free chlorine and various levels of organic chloramines and inorganic contaminants. It is generally assumed that the free chlorine has reacted with all easily oxidizable species present and the remaining free chlorine is likely present in a steady-state equilibrium. Replicate analyses for free chlorine on this type of water should give equivalent results. It is especially important when testing water where free chlorine residual levels are low to observe all precautions that refer to sample cell cleanliness, water temperature and sampling techniques.

At breakpoint
These waters may contain a mixture of free chlorine, chloramines and nuisance residuals depending on water temperature, mixing efficiencies, sampling location and distance beyond the theoretical breakpoint. It is important to note that the water can be in a state of "dynamic equilibrium" and the chemical speciation can change rapidly, especially if one is at or near breakpoint. The chemical speciation can change dynamically in both the Blank cell and the Sample cell. The test must be conducted immediately on these types of samples. Test results may be difficult to replicate on duplicate samples depending on the dynamics of the water. Test results are best used to identify free chlorine trends and to monitor changes due to different mixing efficiencies, sampling locations, temperature changes, increased chlorine feed rates, etc.

In chloramination kinetic studies
These waters will contain a mixture of free chlorine and chloramines depending on water temperature, mixing efficiencies, sampling locations, feed rates for chlorine and ammonia and contact time. It is important to note that the water is in a state of "dynamic equilibrium" and the chemical speciation can change rapidly depending on water conditions. The chemical speciation can change dynamically in both the Blank cell and the Sample cell. The test must be conducted immediately on these types of samples. Test results may be difficult to replicate on duplicate samples depending on the dynamics of the water. Test results are best used to identify free chlorine trends and to monitor changes based on changes in mixing efficiencies, sampling locations, water temperature changes, increased chlorine feed rates, etc.

With other oxidants such as Oxone, permanganate, chlorine dioxide, bromine and iodine
It is assumed that the free chlorine residual has stabilized in the presence of the other oxidants. Replicate analyses for free chlorine on this type of water should give equivalent results. The levels of alternate oxidants that can be present without interference have been tested only in laboratory bench studies (refer to Table 104). Field data for free chlorine in the presence of these oxidants is not available.

Accuracy check

Important Note: *This procedure is only valid for stabilized or equilibrated free chlorine samples.*

Standard additions method (sample spike)
Required for accuracy check:

- Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L
- PourRite Ampule Breaker
- Pipet, TenSette®, 0.1–1.0 mL
- Pipet tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu (if available). Refer to the instrument user manual for specific instructions. Refer to step 9 if a standard additions menu is not available.
3. Enter the average chlorine concentration from the label or certificate that is enclosed with the standard solution ampules.

4. A summary of the standard additions procedure will be displayed. Press **OK** to accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row.
5. Open one standard solution ampule.
6. Use the TenSette Pipet to prepare 3 spiked samples: add 0.1 mL, 0.2 mL, and 0.3 mL of standard to three 10-mL portions of fresh sample.
7. Follow the *Indophenol Method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.
9. If a standard additions menu is not available, calculate the percent recovery:
   - **c.** Put the spiked sample in the cell holder and press **READ**.
   - **d.** Calculate the concentration of chlorine that was added to the sample:

$$\text{mg/L chlorine added} = \frac{0.1\ \text{mL} \times \text{concentration of chlorine standard (mg/L } Cl_2)}{10.1\ \text{mL}}$$

   - **e.** Find the expected result of the spiked sample from the sum of the unspiked sample result plus the concentration of chlorine that was added (step d).
   - **f.** Calculate the percent recovery from the actual (step c) and the expected (step e) results.

Note: *If results are not within acceptable limits (± 10%), be sure that the sample volumes and sample spikes are measured accurately. The sample volumes and sample spikes that are used should agree with the selections in the standard additions menu. If all procedures are followed correctly but the standard additions results are not within acceptable limits, the sample may contain an interference.*

Method performance

In a single laboratory with a standard solution of 3.51 mg/L chlorine (as Cl_2) and a single lot of reagent with a single instrument (DR 5000), a single operator obtained a standard deviation of ± 0.04 mg/L Cl_2.

Summary of method

An ammonia solution at a pH of 8.3 is added to a sample containing free chlorine. The free chlorine is immediately converted into monochloramine (NH_2Cl). The monochloramine is then determined by the indophenol method using Monochlor F Reagent. In this method, the monochloramine reacts with a substituted phenol in the presence of a cyanoferrate catalyst to form an intermediate monoimine compound. The intermediate compound couples with excess substituted phenol to form a green-colored indophenol compound, which is proportional to the amount of free chlorine present in the sample. A sample blank containing Monochlor F Reagent compensates for background color from the reagent and sample. Test results are measured with a spectrophotometer at 655 nm or with a colorimeter at 610 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Freechlor F Reagent Solution	5 drops	50-mL SCDB	2964926
Monochlor F Reagent Pillows	2	100/pkg	2802299

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule Breaker, 2-mL PourRite Ampules	each	2484600
Ampule Breaker Kit, 10-mL Volulette Ampules	each	2196800
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Clippers (shears)	each	2369400
Thermometer, non-mercury, –10 to 225 °C	each	2635700
Wipers, disposable, 28 x 37 cm	188/pkg	2932800

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Edition 7

Chlorine, Total

DOC316.53.01027

USEPA[1] DPD Method[2] **Method 8167**

(0.02 to 2.00 mg/L) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For testing residual chlorine and chloramines in water, wastewater, estuary water and seawater; USEPA-accepted[1] for reporting for drinking and wastewater analyses.

[1] Procedure is equivalent to USEPA method and Standard Method 4500-Cl G for drinking water and wastewater analyses.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 106 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

Samples must be analyzed immediately and cannot be preserved for later analysis
If the test overranges, dilute the sample with a known volume of high quality, chlorine demand-free water and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. Or, analyze samples with high chlorine concentrations directly without dilution by using Method 10070, Chlorine, Total HR, or Method 10250, Chlorine Total, MR.
For chloramination disinfection control, use Method 10172, Chloramine (Mono), Low Range (program number 66) or High Range (program number 67).
After adding reagent a pink color will develop.
The SwifTest Dispenser[1] for Total Chlorine can be used in place of the powder pillow in step 3.
An AccuVac ampule for Blanks can be used as a blank in place of the sample cell in step 2.

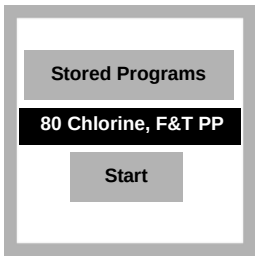
[1] *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
DPD Total Chlorine Reagent powder pillow, 10-mL	1
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
DPD Total Chlorine Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1

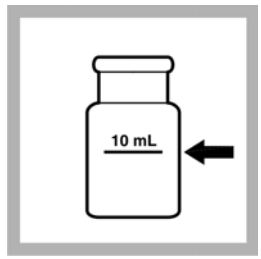
See *Consumables and replacement items* for reorder information.

DPD method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Fill a sample cell with 10 mL of sample.

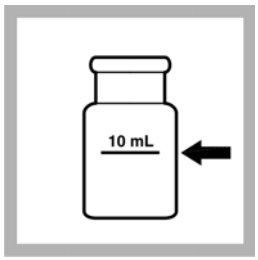
3. Prepared Sample:

Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell.

Swirl the sample cell for 20 seconds to mix.

4. Start the instrument timer.

A three-minute reaction period will begin. Perform steps 5 and 6 during this time period.



5. Blank Preparation:
Fill a second sample cell with 10-mL of sample.

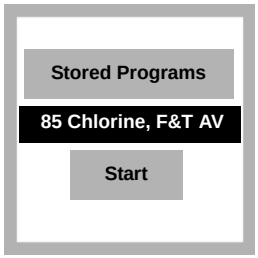
6. Wipe the blank sample cell and insert it into the cell holder.

ZERO the instrument. The display will show:
0.00 mg/L Cl_2

7. Within three minutes after the timer expires, wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L Cl_2.

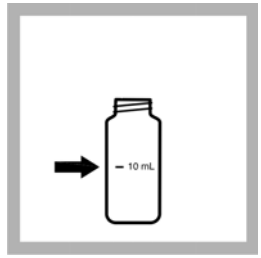
DPD method for AccuVac Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a sample cell with 10-mL of sample.

3. Prepared Sample:

Fill a DPD Total Chlorine Reagent AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely.

4. Quickly invert the Ampul several times to mix. Wipe off any liquid or fingerprints.

5. Start the instrument timer.

A three-minute reaction period will begin. Perform steps 6 and 7 during this time period.

6. Wipe the blank sample cell and insert it into the cell holder.

ZERO the instrument. The display will show: 0.00 mg/L Cl_2.

7. Within three minutes after the timer expires, wipe the AccuVac Ampul and insert it into the cell holder.

READ the results in mg/L Cl_2.

Interferences

Table 107 Interfering substances and levels

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 300 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as $CaCO_3$

Table 107 Interfering substances and levels (continued)

Interfering Substance	Interference Levels and Treatments
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops potassium iodide[1] (30 g/L) to a 25-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops sodium arsenite[1, 2] (5 g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or Highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid[1], 1.000 N) or base (Sodium Hydroxide[1], 1.00 N).

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). Reference the current MSDS for more information on proper disposal of these materials.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark.
- Perform the chlorine analysis immediately.

Accuracy check

Required for accuracy check:

- Chlorine Voluette® Ampule Standard, 25–30 mg/L Cl_2.
- TenSette Pipet and tips
- Ampule Breaker

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.

2. Select Options>More>Standard Additions from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a LR Chlorine Voluette Ampule Standard, 25–30 mg/L Cl_2.
5. Prepare three sample spikes. Fill three mixing cylinders with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.

   Note: *For AccuVac® Ampuls, fill three mixing cylinders with 50-mL of sample and spike with 0.4 mL, 0.8 mL and 1.2 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers*. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by pressing Read. Each addition should reflect approximately 100% recovery.*

6. Analyze each sample spike as described in the procedure above, starting with the smallest sample spike. Accept each standard additions reading by pressing Read. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press GRAPH to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press IDEAL LINE to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
80	1.25 mg/L Cl_2	1.23–1.27 mg/L Cl_2	0.02 mg/L Cl_2
85	1.25 mg/L Cl_2	1.21–1.29 mg/L Cl_2	0.02 mg/L Cl_2

Summary of method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid and/or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives. The combined chlorine oxidizes iodide in the reagent to iodine. The iodine and free chlorine reacts with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test and a total chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
DPD Total Chlorine Reagent Powder Pillows, 10-mL	1	100/pkg	2105669
OR			
DPD Total Chlorine Reagent AccuVac® Ampuls	1	25/pkg	2503025

Required apparatus

Description	Quantity	Unit	Catalog number
AccuVac snapper	1	each	2405200
Beaker, 50-mL	1	each	50041H

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL Pour-Rite® Ampule, 25–30 mg/L	20/pkg	2630020
Chlorine Standard Solution, 2-mL PourRite® Ampules, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette® Ampules, 50–75 mg/L	16/pkg	1426810
Voluette Ampule breaker 10 mL	each	2196800
PourRite Ampule breaker 2-mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Beakers, 50 mL	each	50041H
Chlorine Demand-Free Water	500 mL	2641549
Cylinder, mixing, 25 mL	each	2088640
Cylinder, mixing, 50 mL	each	189641
Deionized Water	4L	27256
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sodium Hydroxide, 1 N	100 mL	104532
Sulfuric Acid, 1 N	100 mL	127032
SwifTest Dispenser for Total Chlorine	each	2802400
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
pH Paper, 0–14 pH range	100/pkg	2601300
AccuVac, vials for sample blanks	25/pkg	2677925
DPD Total Chlorine Reagent Powder Pillows, 10-mL	1000/pkg	2105628
DPD Total Chlorine Reagent Powder Pillows, 10-mL	300/pkg	2105603
DPD Total Chlorine Reagent, 10 mL, SwifTest Dispenser refill vial	250 tests	2105660
SpecCheck Secondary Standard Kit, Chlorine DPD, 0 - 2.0 mg/L Set	each	2635300

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Edition 7

Chlorine, Total

DOC316.53.01030

DPD Rapid Liquid Method[1] **Method 10060**

(0.02 to 2.00 mg/L) **Pour-Thru Cell**

Scope and Application: For treated water.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 108 Instrument-specific information

Instrument	Pour-thru Kit	Cell orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

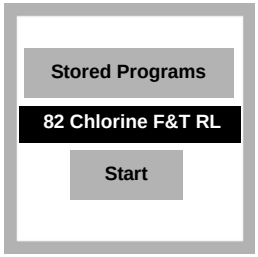
Analyze samples immediately. Do not preserve for later analysis.
Refer to the instrument User Manual for Pour-Thru cell and module assembly and installation.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel. Refer to *Reagent preparation*
Make sure the Pour-Thru cell is completely seated in the sample cell compartment.

Collect the following items:

Description	Quantity
DPD Indicator Powder	varies
Total Chlorine Indicator Solution	1 mL
Total Chlorine Buffer Solution	1 mL
Cylinder, glass, mixing, 100-mL	1
Dispenser, Adjustable Volume	2
Pour-Thru Module and Cell (See instrument-Specific Information)	1

See *Consumables and replacement items* for reorder information.

DPD rapid liquid method for Pour-Thru Cell

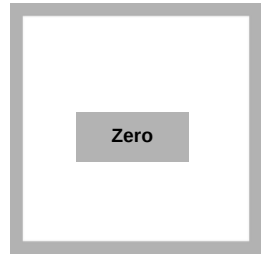


1. Select the test.

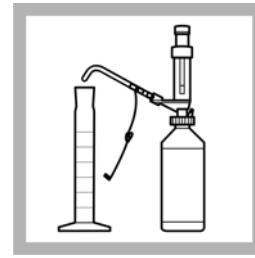
Insert an adapter if required (*Instrument-specific information*).

Refer to *Instrument-specific information* for Pour-thru cell orientation.

2. Pour approximately 50 mL of sample into the Pour-Thru Cell.

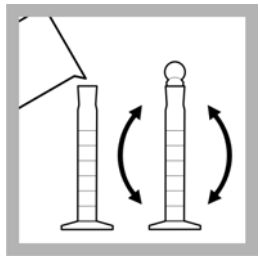


3. When the flow stops, **ZERO** the instrument. The display will show: 0.00 mg/L Cl_2.



4. Add 1.0 mL of Total Chlorine Buffer Solution to a clean, dry 100-mL glass mixing cylinder using the Repipet Jr. Dispenser.

5. Add 1.0 mL of prepared Total Chlorine Indicator Solution to the same mixing cylinder using the Repipet Jr. Dispenser. Swirl to mix the reagents. Proceed to step 6 immediately.

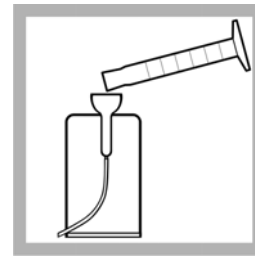


6. Carefully fill the mixing cylinder to the 80-mL mark with sample. Stopper the cylinder and gently invert it twice to mix. Proceed to step 7 immediately.

7. Start the instrument timer.

A two-minute reaction period will begin.

Complete steps 8 and 9 within two minutes after the timer expires.



8. When the timer expires, fill the funnel of the Pour-Thru Cell with the reacted sample from the mixing cylinder.

It is not necessary to pour the entire sample into the Pour-Thru Cell; approximately half of the sample may be discarded.

DPD rapid liquid method for Pour-Thru Cell (continued)

9. After the flow stops, **READ** the results in mg/L Cl_2.

10. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use.

Reagent preparation

The Total Chlorine Indicator Solution must be prepared before use. Using a powder funnel, add the contents of one 24 g bottle of DPD Powder to one 473-mL bottle of Total Chlorine Indicator Solution*. Invert several times and swirl until the powder is completely dissolved. A pale pink color may develop, but should not affect results.

This solution will give accurate results for at least one month after mixing when stored at 20–25 °C (68–77 °F). Write the date of preparation on the Indicator Solution Bottle. Discard any remaining solution after one month. Use of this reagent after one month may result in high reagent blanks and low values at high concentration. Do not combine fresh reagent with previously mixed reagent.

Interferences

Table 109 Interfering substances and levels

Interfering substance	Interference levels and treatments
Alkalinity	Greater than 700 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels.
Hardness	Levels below 1000 mg/L as $CaCO_3$ will not interfere.
Hexavalent Chromium	Levels greater than 1 mg/L will cause a positive interference.
Iodine, I_2	Interferes at all levels.
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid[1]. **2.** Add 9 drops Potassium Iodide (30 g/L)[1] to an 80-mL sample. **3.** Mix and wait 1 minute. **4.** Add 9 drops Sodium Arsenite[1, 2] (5 g/L) and mix. **5.** Analyze the treated sample as described in the procedure above. **6.** Subtract the result of this test from the original analysis to obtain the correct concentration.
Ozone	Interferes at all levels.

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). Refer to the current MSDS for safe handling and disposal instructions.

Sampling and storage

- Samples must be analyzed immediately and cannot be preserved for later analysis.
- A common testing error is introduced if the analyst does not obtain a representative sample. If sampling from a tap, let the water flow for at least five minutes to make sure that it is a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.
- Avoid plastic containers since these may have a chlorine demand.
- Pre-treat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized water. If sample containers are rinsed thoroughly with deionized water after use, only occasional pretreatment is necessary. A pre-treated BOD bottle with a ground-glass stopper is an ideal sample container for chlorine collection.

Treating analysis labware

Glassware used in this test must be chlorine demand-free. Fill the 100-mL mixing cylinder and sample container with a dilute solution of chlorine bleach prepared by adding 1 mL of commercial bleach to 1 liter of water. Soak in this solution at least one hour. After soaking, rinse thoroughly with deionized water and allow to dry before use. If the mixing cylinder is thoroughly rinsed with deionized water and allowed to dry after each use, only occasional pretreatment is necessary. Do not use the same mixing cylinder for Free and Total Chlorine analysis.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes. Rinse several times with deionized water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N Sulfuric Acid followed by several rinsings with deionized water.

Accuracy check

Required for accuracy check

- Chlorine Voluette® Ampule Standard Solution, 50 to 75-mg/L Cl_2
- TenSette® Pipet and tips
- Ampule Breaker

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **Options>More>Standard Additions** from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.Open a Chlorine Voluette Ampule Standard Solution, 50 to 75-mg/L Cl_2.
4. Prepare three sample spikes. Use the TenSette Pipet to add 0.3, 0.6 and 0.9 mL of standard to three 80-mL samples, respectively. Swirl gently to mix.

5. Analyze each sample spike as described in the procedure above, starting with the 0.3 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.

6. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Point of curve	Concentration
82	1.18 mg/L Cl_2	1.17–1.19 mg/L Cl_2	Entire range	0.02 mg/L Cl_2

Summary of method

Chlorine can be present in water as free available chlorine and as combined available chlorine. Both forms can exist in the same water and can be determined together as the total available chlorine. Free chlorine is available as hypochlorous acid and/or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives. The combined chlorine oxidizes iodide in the reagent to iodine. The iodine reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator along with free chlorine present in the sample to form a pink color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test and a total chlorine test. Subtract the free chlorine results from the results of the total chlorine test to obtain combined chlorine. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Rapid Liquid Total Chlorine Reagent Set, includes:	—	—	2557000
DPD Indicator Powder, 24 g	varies	each	2297255
Total Chlorine Indicator Solution	1 mL	473 mL	2263411
Total Chlorine Buffer Solution	1 mL	473 mL	2263511

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, mixing graduated, 100-mL, glass	1	each	2636342
Dispenser, Adjustable volume, 1.0-mL – 5.0 mL	2	each	2563137
Funnel, powder	1	each	2264467

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, Voluette® Ampule, 50–75 mg/L, 10-mL	16/pkg	1426810
OR		
Chlorine Standard Solution, Pour-Rite® Ampule, 50–75 mg/L, 2-mL	20/pkg	1426820

Recommended standards

Description	Unit	Catalog number
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
pH Paper, 0–14 pH range	100/pkg	2601300
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50 pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Potassium Iodide, 30 g/L	100 mL	34332
PourRite Ampule breaker 2-mL	each	2484600
Sodium Arsenite, 5 g/L	100 mL	104732
Sulfuric Acid, 1 N	100 mL	127032
Sulfuric Acid, 5.25 N	1000 mL	244953
Voluette Ampule Breaker	each	2196800

[1] Other sizes are available

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Edition 7

Chlorine, Total

DOC316.53.01028

DPD Method[1] **Method 10101**

(0.09 to 5.00 mg/L) **Test ‘N Tube™ Vials**

Scope and Application: For testing higher levels of total (free plus combined) chlorine in drinking water, treated wastewater, cooling water or industrial process water

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 110 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

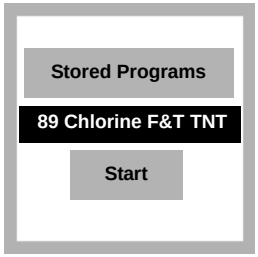
For DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield before performing this test.
Analyze samples immediately. Do not preserve samples for later analysis.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
For chloramination disinfection control, use Method 10172, Chloramine (Mono), High Range.
After adding sample to the Test ‘N Tube™, a pink color will develop if free chlorine is present.

Collect the following items:

Description	Quantity
Light Shield (see *Instrument-specific information*)	1
Test ‘N Tube™ DPD Total Chlorine Reagent	1

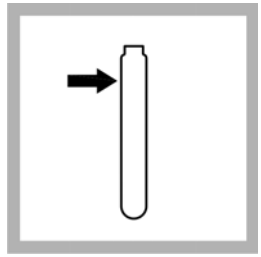
See *Consumables and replacement items* for reorder information.

DPD method for Test ‘N’Tubes



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

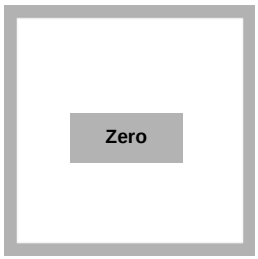


2. Blank Preparation:

Fill an empty Test ‘N Tube vial to the top of the label with sample.

3. Wipe the outside of the vial to remove fingerprints and other marks.

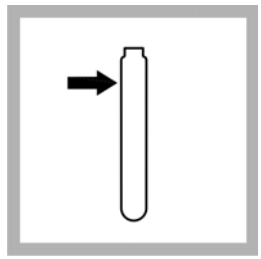
4. Insert the blank into the cell holder.



5. ZERO the instrument.

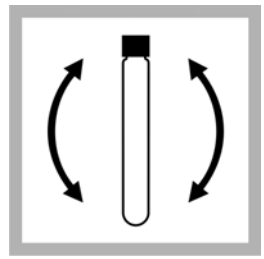
The display will show:

0.00 mg/L Cl_2



6. Prepared Sample:

Remove the cap from a Total Chlorine DPD Test ‘N Tube™. Add 10 mL of sample to the tube. (Fill the vial to the top of the label.)



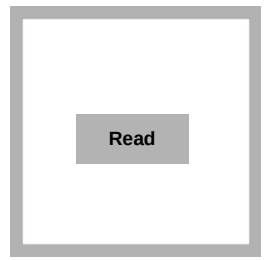
7. Cap and slowly invert at least 10 times to dissolve the powder. (Ten inversions should take at least 30 seconds. One inversion equals turning the vial upside down, then returning it to an upright position.)

8. Start the instrument timer.

A three-minute reaction period will begin.

9. When the timer expires, wipe the outside of the vial that contains the prepared sample.

10. Insert the sample in the cell holder.



11. READ the results in mg/L Cl_2.

Interferences

Table 111 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatment
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N Sodium Hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 300 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as $CaCO_3$
Iodine, I_2	Interferes at all levels
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide[1] (30 g/L) to a 25-mL sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops Sodium Arsenite[1], [2] (5 g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result of this test from the original analysis to obtain the correct chlorine concentration.
Ozone, O_3	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid[1], 1.000 N) or base (Sodium Hydroxide[1], 1.00 N).

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See the current reagent MSDS for safe disposal instructions.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Free chlorine and combined chlorine are strong oxidizing agents and are unstable in natural waters. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- A common error in testing for chlorine is obtaining an unrepresentative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Accuracy check

Required for accuracy check:

- Chlorine PourRite® Ampule Standard, 50-75 mg/L Cl_2
- TenSette Pipet and tips
- Ampule Breaker

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Chlorine PourRite Ampule Standard, 50-75 mg/L Cl_2.
5. Use the TenSette® Pipet to add 0.1 mL of standard to a 10-mL sample and mix thoroughly.
6. Analyze the standard addition sample as described in the procedure above. Accept the standard additions reading. The addition should reflect approximately 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
89	2.68 mg/L Cl_2	2.63–2.73 mg/L Cl_2	0.03 mg/L Cl_2

Summary of method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid and/or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives.

Free or combined chlorine oxidizes iodide in the reagent to iodine. The iodine and chlorine react with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color, which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test and a total chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Test ‘N Tube™ DPD Total Chlorine Reagent	1	50/pkg	2105645

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
PourRite Ampule breaker 2-mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
pH Paper, 0–14 pH range	100/pkg	2601300
Voluette Ampule breaker 10 mL	each	2196800
Test Tube Rack	each	1864100
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite 5 g/L	100 mL	104732
Sodium Hydroxide, 1.00 N	100 mL	104532
Sulfuric Acid, 1.000 N	100 mL	127032
Chlorine Standard Solution, 10-mL Voluette® Ampules, 50–75 mg/L	16/pkg	1426810
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020

[1] Other sizes are available

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Edition 7

Chlorine, Total

DOC316.53.01029

USEPA DPD Method[1] **Method 10070**

HR (0.1 to 10.0 mg/L as Cl_2) **Powder Pillows**

Scope and Application: For testing higher levels of total chlorine (free and combined) in drinking water, cooling water and industrial process waters

[1] *USEPA accepted for reporting drinking water analyses. Procedure is equivalent to USEPA, Standard Method 4500-Cl-G for Drinking Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 112 Instrument-specific information

Instrument	Sample cell	Cell orientation	Adapter
DR 6000	4864302	Orientation key toward user	
DR 5000	4864302	Orientation key toward user	—
DR 3900	4864302	Orientation key inserted in adapter slot	LZV846 (A)
DR 3800, DR 2800, DR 2700	5940506	1-cm (flat) path aligned with the arrow on the adapter	LZV585 (B)

Before starting the test:

Analyze samples immediately. Do not preserve for later analysis.
If the chlorine concentration is less than 2 mg/L, use Method 8167, program number 80.
After adding the reagent, a pink color will develop if chlorine is present.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

Collect the following items:

Description	Quantity
DPD Total Chlorine Reagent Powder Pillows	1
Sample Cell (See Instrument Specific Information)	1

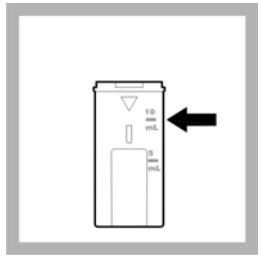
See *Consumables and replacement items* for reorder information.

DPD method for powder pillows

1. Select the test.

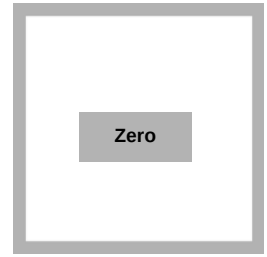
Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill the sample cell to the 5-mL line with sample.

3. Wipe the cell and insert it into the cell holder (*Instrument-specific information*.

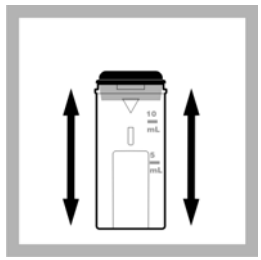


4. **ZERO** the instrument.

The display will show:

0.0 mg/L Cl_2

5. Remove the cell and add the contents of one DPD Total Chlorine powder pillow for 25-mL samples to the sample.



6. Cap and shake the cell about 20 seconds to dissolve reagent.

7. Start the instrument timer.

A 3-minute reaction period will begin.

8. Insert the prepared sample into the *Instrument-specific information*

READ the results in mg/L Cl_2.

Interferences

Table 113 Interfering substances and levels

Interfering substance	Interference levels and treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N Sodium Hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere

Table 113 Interfering substances and levels (continued)

Interfering substance	Interference levels and treatments
Iodine, I_2	Interferes at all levels
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid[1]. **2.** Add 2 drops Potassium Iodide[1] (30 g/L) to a 5-mL sample. **3.** Mix and wait 1 minute. **4.** Add 2 drops Sodium Arsenite[1, 2] (5 g/L) and mix. **5.** Analyze the treated sample as described in the procedure above. **6.** Subtract the result of this test from the original analysis to obtain the correct concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid[1]) or base (Sodium Hydroxide[1]).

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). Refer to the current MSDS for safe handling and disposal instructions.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Free and combined chlorine are strong oxidizing agents and react rapidly with various compounds. Many factors such as sunlight, pH, temperature and sample composition will influence decomposition of chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over to the free chlorine test, monochloramine could interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.
- A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 5-mL mark. Proceed with the chlorine test immediately.

Accuracy check

Required for accuracy check:

- Chlorine PourRite® Ampule Standard, 50–75 mg/L
- TenSette Pipet and tips

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.

3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Chlorine PourRite® Ampule Standard, 50–75 mg/L*.
5. Prepare three sample spikes. Fill three mixing cylinders with 5-mL of sample. Using the TenSette® Pipet*, add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by pressing Read. Each addition should reflect approximately 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Point of Curve	Concentration
88	5.4 mg/L Cl_2	5.3–5.5 mg/L	Entire range	0.1 mg/L Cl_2

Use Method 8167 to test chlorine concentrations at levels typically less than 2 mg/L or Method 8370 to test chlorine concentrations at levels typically less than 500 µg/L.

Summary of method

The range of analysis using the DPD method for total chlorine can be extended by adding more indicator in proportion to sample volume. Thus, a larger fill powder pillow of DPD Total Chlorine Reagent is added to a 5-mL sample portion.

The combined chlorine oxidizes iodide in the reagent to iodine. The iodine reacts with DPD (N, N-diethyl-p-phenylenediamine) along with free chlorine present in the sample to form a pink color which is proportional to the total chlorine concentration. Test results are measured at 530 nm.

Consumables and replacement items

Required Reagents

Description	Quantity/Test	Unit	Catalog number
DPD Total Chlorine Reagent Powder Pillows for 25-mL samples	1	100/pkg	1406499

Recommended Standards

Description	Unit	Catalog Number
Chlorine Standard Solution, 2-mL PourRite® Ampules, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette ampules, 50–75 mg/L	16/pkg	1426810

* See *Optional reagents and apparatus*.

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule breaker, Voluette, 10 mL	each	2196800
Ampule breaker, PourRite, 2-mL	each	2484600
Cylinder, mixing, 25 mL	each	189640
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
pH Paper, 0–14 pH range	100/pkg	2601300
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sodium Hydroxide, 1 N	100 mL	104532
Sulfuric Acid, 1 N	100 mL	127032
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
DPD Total Chlorine Reagent Powder Pillows, 25-mL	1000/pkg	1406428

[1] Other sizes are available

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
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Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

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Edition 7

Chlorine, Total

DOC316.53.01032

USEPA DPD Method[1] **Method 10014**

ULR (2 to 500 µg/L as Cl_2) **Pour-Thru Cell and OriFlo™ Filtration**

Scope and Application: For testing trace levels of chlorine and chloramines in treated domestic and industrial wastewater; USEPA accepted for reporting for wastewater analysis[2]

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

[2] U.S. Patent 5,362,650 covers the procedure. U.S. Patent 5,549,816 covers the OriFlo™ Filtration System.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 114 Instrument-specific information

Instrument	Pour-thru Kit	Cell orientation[1]	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

[1] Align for long path

Before starting the test:

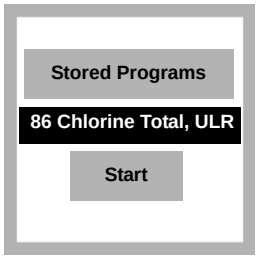
Analyze samples immediately. Samples containing chlorine cannot be preserved for later analysis.
A reagent blank value for a combined lot of indicator/buffer reagent solutions should be determined at least once a day. If sample color or turbidity fluctuates frequently during the day, determine a reagent blank for each sample. Refer to Treating Analysis Labware on page 7.
The reagent blank value is normally less than 5 µg/L. If the value is greater than 5 µg/L, an interfering substance may be present in the blanking water or the DPD Indicator may be degrading. If there is doubt about the reagents, repeat the reagent blank determination using chlorine-demand-free water for the sample. Blanks up to 5 µg/L may be used.
Use a new filter for each test. Using an unspecified filter may give low analysis results or inability to filter the required volume.
Ampules contain more than 1.0 mL of solution for ease of transfer. Discard excess reagent in the ampule.
Refer to the instrument User Manual for Pour-Thru cell and module assembly and installation.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
Use forceps to handle filters.
Make sure the Pour-Thru cell is completely seated in the sample cell compartment.

Collect the following items:

Description	Quantity
ULR Chlorine Buffer Solution, 1.5-mL ampules	1 mL
DPD Indicator Solution for ULR Chlorine, 1.5-mL ampules	1 mL
Blanking Reagent for ULR Chlorine	1 mL
Membrane Filters, 3-micron, 25-mm	1
OriFlo Assembly	1
Beaker, 250 mL	1
Cylinder, graduated mixing, 50-mL.	1
Pipet, TenSette®, 0.1 to 1.0 mL	1
Pipet Tips	2
Deionized water	Varies
Ampule Breaker	1
Pour-Thru Module and cell	1

See *Consumables and replacement items* for reorder information.

DPD method for Pour-Thru Cell

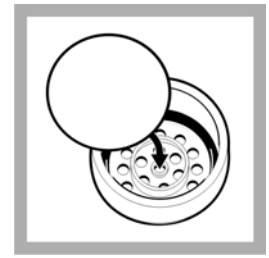


1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

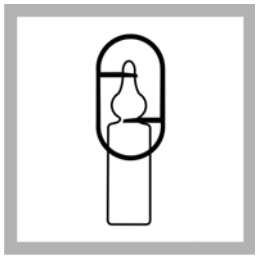
2. Flush the Pour-Thru cell with 50 mL of deionized water.

3. Unscrew the cap from the OriFlo™ plunger assembly. Be sure that the O-ring is properly seated in the cap.

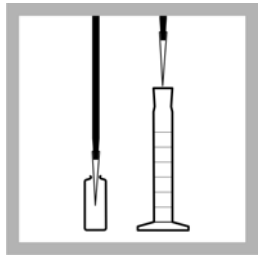


4. Install a new, 3-micron filter (white) into the cap well. Wet the filter with a few drops of deionized water. Reassemble and hand-tighten the cap onto the plunger.

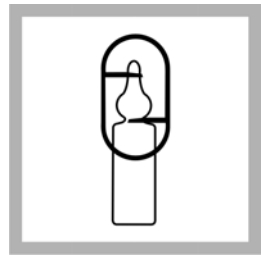
DPD method for Pour-Thru Cell (continued)



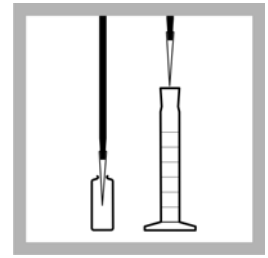
5. Open one ULR Chlorine Buffer Solution Ampule.



6. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of buffer from the ampule to a clean, treated 50-mL graduated mixing cylinder.

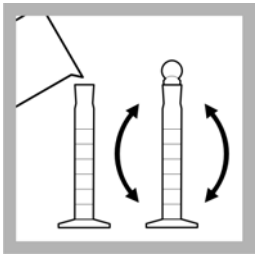


7. Open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine.



8. Use a TenSette Pipet and a clean tip to transfer 1.0 mL of indicator from the ampule to the graduated mixing cylinder. Swirl to mix.

Proceed to step 9 within one minute.

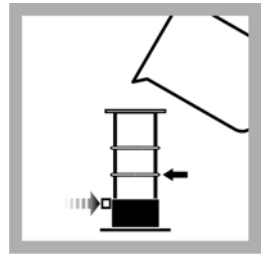


9. Prepared Sample: Avoiding extra agitation, carefully fill the cylinder to the 50-mL mark with sample. Stopper the cylinder. Gently invert it twice to mix.

10. Press **TIMER>OK**.

A three-minute reaction time will begin. Perform steps 11–16 during this period.

Measure the reacted sample 3–6 minutes after mixing the sample and reagents. If less than three minutes elapses, the reaction with chloramines may be incomplete. A reading after six minutes may result in higher reagent blank values.

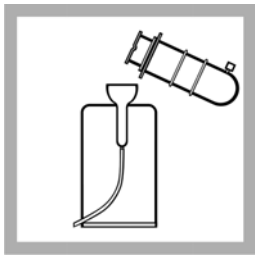


11. Push the valve button on the OriFlo™ barrel assembly in ("closed" position). Place the barrel assembly into its stand. Pour approximately 50 mL of the original sample into the barrel.

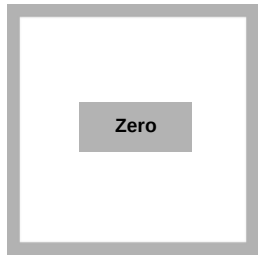
The lower ring on the barrel assembly represents about a 50-mL volume.

12. Insert the plunger into the barrel and slowly push the plunger down with even pressure, until the plunger is fully seated.

DPD method for Pour-Thru Cell (continued)



13. Pour the filtered sample from the plunger reservoir into the Pour-Thru Cell.

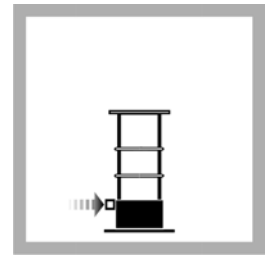


14. When the flow stops, **ZERO** the instrument.

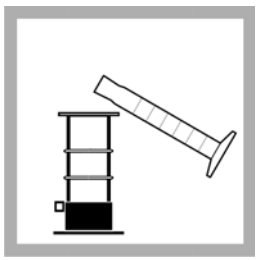
The display will show: 0 µg/L Cl_2.

15. Pull the barrel valve button out to the “open” position. Pull the plunger up to separate it from the barrel assembly. Discard the remaining unfiltered sample.

A new membrane may be required for very turbid samples. Alternatively, use a second Quick Filter unit with a new membrane filter installed.

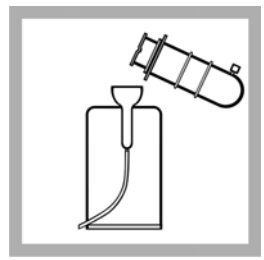


16. Push the barrel valve button to the “closed” position. Place the barrel assembly into its stand.



17. When the timer expires, pour the contents of the mixing cylinder into the barrel.

18. Insert the plunger into the barrel and slowly push the plunger down with even pressure, until the plunger is fully seated.



19. Pour the filtered, reacted sample from the plunger reservoir into the Pour-Thru Cell.

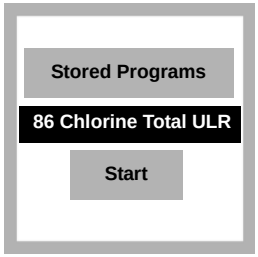
READ the results in µg/L chlorine.

If a dechlorinating agent (e.g., sulfite or sulfur dioxide) is present, the sample result, corrected for the reagent blank, will read “0” or a slightly negative value.

20. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use.

Subtract the reagent blank value (See *Determining the reagent blank value*) from the sample value obtained in step 19.

Determining the reagent blank value



1. Select the test.

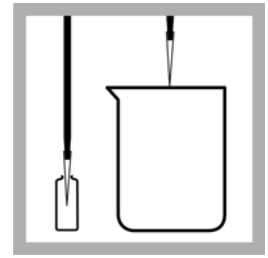
Make sure that the reagent blank setting is off.

See the user manual for information.

2. Install the Pour-Thru module.

Flush the Pour-Thru cell with 50 mL of deionized water.

3. Collect about 100 mL of deionized or tap water in a clean, 250-mL beaker.

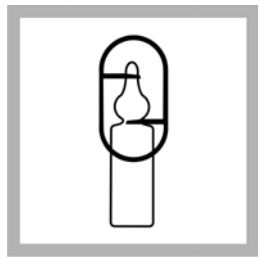


4. Use a TenSette® Pipet to add 1.0 mL of Blanking Reagent to the beaker. Swirl several times to mix.

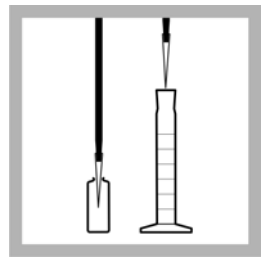
The Blanking Reagent removes chlorine and chloramines from the water.

Note: *The solution from step 4 will be used in Step 10.*

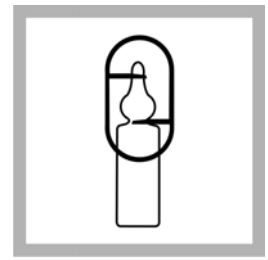
5. Access the general timer and set it for five minutes. Start the timer.



6. After the timer expires, open one ampule of ULR Chlorine Buffer Solution.

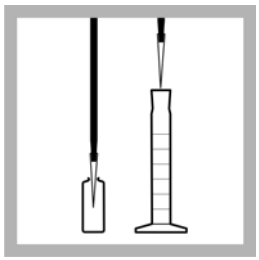


7. Use a TenSette Pipet and a clean tip to transfer 1.0 mL of buffer from the ampule a clean 50 mL mixing graduated cylinder.

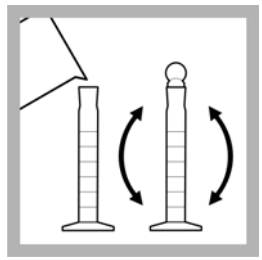


8. Open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine.

Determining the reagent blank value (continued)



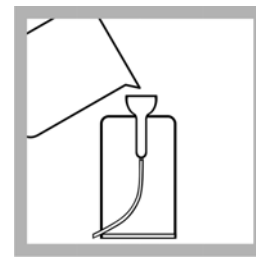
9. Use a TenSette Pipet and a clean tip to transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix the reagents. Proceed to step 10 within one minute.



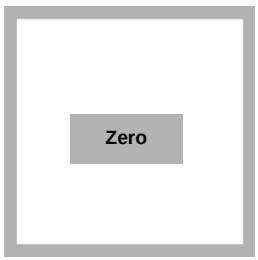
10. Fill the cylinder to the 50-mL mark with dechlorinated water from step 4. Cap and invert twice to mix. Save the remaining water for step 12.

11. Start the instrument timer.

A three-minute reaction time will begin.



12. During the reaction period, flush the Pour-Thru Cell with the remainder of original dechlorinated water from step 10.



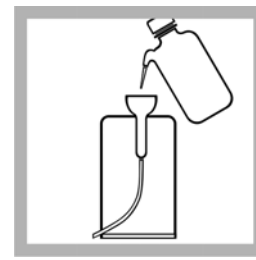
13. When the flow stops, **ZERO** the instrument.

The display will show: 0 µg/L Cl_2.

14. When the timer expires, pour the contents of the cylinder into the Pour-Thru Cell. **READ** the results in µg/L chlorine.

15. Use this value to correct the sample result obtained in this procedure.

See the user manual for details on saving the reagent blank value.



16. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use.

Interferences

Table 115 Interfering substances and levels

<table>
<tr><th>Interfering substance</th><th>Interference levels and treatments</th></tr>
<tr><td>Bromine, Br_2</td><td>Interferes at all levels.</td></tr>
<tr><td>Chlorine Dioxide, ClO_2</td><td>Interferes at all levels</td></tr>
<tr><td>Chloramines, organic</td><td>May interfere</td></tr>
<tr><td>Copper, Cu^{2+}</td><td>Greater than 1000 µg/L</td></tr>
<tr><td>Iodine, I_2</td><td>Interferes at all levels.</td></tr>
<tr><td>Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})</td><td>1. Adjust sample pH to 6–7 with 1.0 N Sulfuric Acid[1].
2. Add 9 drops Potassium Iodide (30 g/L)[1] to an 80-mL sample.
3. Mix and wait 1 minute.
4. Add 9 drops Sodium Arsenite[1, 2] (5 g/L) and mix.
5. Analyze the treated sample as described in the procedure above.
6. Subtract the result of this test from the original analysis to obtain the correct concentration.</td></tr>
<tr><td>Nitrite, NO_2^- (uncommon in clean waters)</td><td><table><tr><th>mg/L nitrite</th><th>Apparent µg/L chlorine</th></tr><tr><td>2.0 mg/L</td><td>3 µg/L</td></tr><tr><td>5.0 mg/L</td><td>5 µg/L</td></tr><tr><td>10.0 mg/L</td><td>7 µg/L</td></tr><tr><td>15.0 mg/L</td><td>16 µg/L</td></tr><tr><td>20.0 mg/L</td><td>18 µg/L</td></tr></table></td></tr>
<tr><td>Ozone</td><td>Interferes at all levels.</td></tr>
<tr><td>Peroxides</td><td>May interfere</td></tr>
<tr><td>Extreme sample pH or highly buffered samples</td><td>Adjust to pH 6–7</td></tr>
</table>

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). Refer to the current MSDS for safe handling and disposal instructions.

Sample collection, storage and preservation

- Analyze samples for chlorine immediately after collection. Chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.
- Avoid plastic containers. These may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour.
- Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no head space (air) above the sample.

- Perform the chlorine analysis immediately.

Treating analysis labware

Glassware used in this test must be chlorine demand-free. Fill the mixing cylinder and sample container with a dilute solution of chlorine bleach prepared by adding 1 mL of commercial bleach to 1 liter of water. Soak in this solution at least one hour. After soaking, rinse thoroughly with deionized water and allow to dry before use.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes. Rinse several times with deionized water.

Cleaning the Pour-Thru cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N Sulfuric Acid* followed by several rinsings with deionized water.

Accuracy check

Required for accuracy check:

- Low Range Chlorine Voluette® Ampule Standard Solution, 25 to 30-mg/L (25,000 to 30,000 µg/L) Cl_2
- TenSette® Pipet and Pipet Tips
- Ampule Breaker

Standard additions method (samples spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Default values for standard concentration, sample volume, and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Snap the top off a Low Range Chlorine Voluette® Ampule Standard Solution, 25 to 30-mg/L (25,000 to 30,000 µg/L).
5. Prepare three sample spikes. Use the TenSette® Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 50-mL samples, respectively. Swirl gently to mix.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

* See *Optional reagents and apparatus*.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Portion of Curve	Concentration
86	295 µg/L Cl_2	290–300 µg/L Cl_2	Entire range	17 µg/L Cl_2

Summary of method

Several modifications to the normal DPD chlorine method are necessary to measure trace levels of chlorine. The Pour-Thru Cell must be used in the spectrophotometer. Liquid reagents are also required. The reproducible optics of the Pour-Thru Cell give more stable readings than is possible with movable sample cells, resulting in more stable measurements.

It is essential that interfering sample turbidity is removed using a 3-micron membrane filter. To avoid chlorine loss, the filtration is done after reacting the DPD with the chlorine in the sample. The filter used has been specifically selected to avoid retention of the colored product. Sample color is compensated by zeroing the spectrophotometer on a filtered sample.

The reagents are packaged in ampules and sealed under argon gas to ensure stability. Use of liquid reagents eliminates any slight turbidity that might be caused by using powdered reagents. Due to the possible oxidation of the reagents (which could give a positive chlorine reading in the blank), a reagent blank must be determined at least once a day for each lot of reagent used. This reagent blank value is subtracted from the sample result and the corrected value is the actual chlorine concentration. Test results are measured at 515 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
ULR Chlorine Reagent Set (approximately 20 tests), includes:	—	—	2563000
ULR Chlorine Buffer Solution, 1.5-mL ampules	1 mL	20/pkg	2493120
DPD Indicator Solution for ULR Chlorine, 1.5-mL ampules	1 mL	20/pkg	2493220
Blanking Reagent for ULR Chlorine	1 mL	29 mL	2493023
Water deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
ULR Chlorine Apparatus Set, includes:	—	—	2595600
Membrane Filters, 3-micron, 25-mm	1	25/pkg	2594025
OriFlo™ Assembly	1	each	4966000
Beaker, 250-mL	1	each	50046H
Breaker, ampule	1	each	2484600
Cylinder, graduated mixing, 50-mL	1	each	189641
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	2	50/pkg	2185696

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, Voluette® Ampule, 25–30 mg/L, 2-mL	20/pkg	2630020

Optional reagents and apparatus

Description	Unit	Catalog number
Forceps, flat square tips, 115 mm	each	1453700
Potassium Iodide, 30 g/L	100 mL MDB	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sulfuric Acid, 1 N 100 mL	100 mL MDB	127032
Sulfuric Acid, 5.25 N	1000 mL	244953

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Edition 7

Chlorine, Total

DOC316.53.01031

USEPA[1] DPD Method[2] **Method 8370**

ULR (2 to 500 µg/L as Cl_2) **Pour-Thru™ Cell**

Scope and Application: For detecting trace levels of chlorine and chloramines in clean waters relatively free of color and turbidity; USEPA accepted for reporting for drinking water analysis.

[1] USEPA accepted

[2] U.S. Patent 5,362,650

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 116 Instrument-specific information

Instrument	Pour-thru Kit	Cell orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

Analyze samples immediately. Samples containing chlorine cannot be preserved for later analysis.
A reagent blank value for a combined lot of indicator/buffer reagent solutions should be determined at least once a day. If sample color or turbidity fluctuates frequently during the day, determine a reagent blank for each sample.
Ampules contain more than 1.0 mL of solution for ease of transfer. Discard excess reagent in the ampule.
Refer to the instrument User Manual for Pour-Thru cell and module assembly and installation.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel. Refer to *Treating analysis labware*.

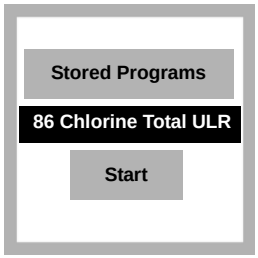
Collect the following items:

Description	Quantity
ULR Chlorine Buffer Solution, 1.5-mL ampules	1 mL
DPD Indicator Solution for ULR Chlorine, 1.5-mL ampules	1 mL
Blanking Reagent for ULR Chlorine	1 mL
Beaker, 250 mL	1
Cylinder, graduated mixing, 50-mL.	1
Pipet, TenSette®, 0.1 to 1.0 mL	1
Pipet Tips for TenSette Pipet	2

Collect the following items: (continued)

Description	Quantity
Pour-Thru Module and cell (See Instrument Specific Information)	1
Ampule Breaker	1
See *Consumables and Replacement Items* for reorder information.	

DPD method for Pour-Thru Cell



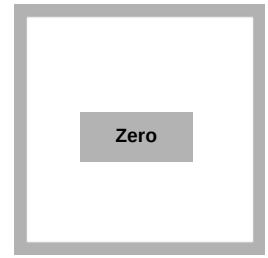
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

2. Pour at least 50 mL of sample into the Pour-Thru Cell.

3. When the flow stops, start the instrument timer.

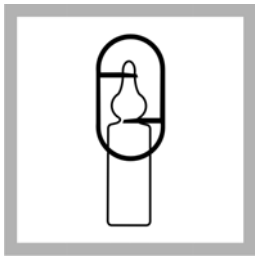
A three-minute reaction period will begin. This time allows turbidity or solids to settle and ensures a stable reading.



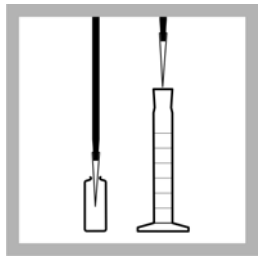
4. When the timer expires, **ZERO** the instrument.

The display will show:

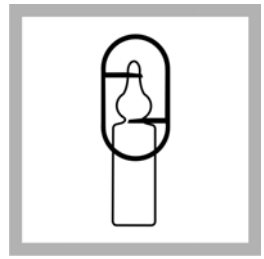
0 µg/L



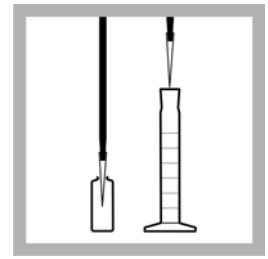
5. Open one ULR Chlorine Buffer Solution Ampule.



6. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of buffer from the ampule to a clean, treated 50-mL graduated mixing cylinder.



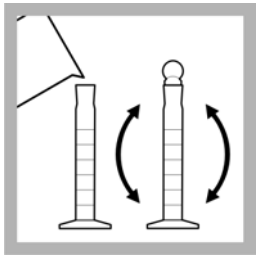
7. Open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine.



8. Use a TenSette Pipet and a clean tip to transfer 1.0 mL of indicator from the ampule to the graduated mixing cylinder. Swirl to mix.

Proceed to step 9 within one minute.

DPD method for Pour-Thru Cell (continued)



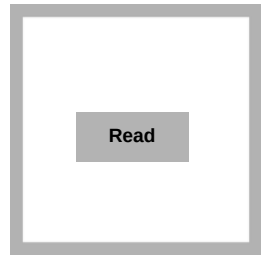
9. Prepared Sample: Avoiding extra agitation, carefully fill the cylinder to the 50-mL mark with sample. Stopper the cylinder. Gently invert it twice to mix.

10. Start the instrument timer.

A three-minute reaction time will begin.

Measure the reacted sample 3–4 minutes after mixing the sample and reagents. If less than three minutes elapses, the reaction with chloramines may be incomplete. A reading after four minutes may result in higher reagent blank values.

11. Pour the contents of the graduated mixing cylinder into the Pour-Thru cell.

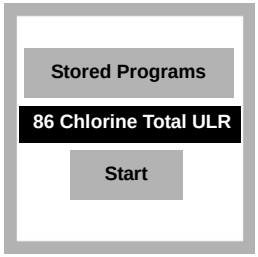


12. When the timer expires, **READ** the results in µg/L chlorine.

If a dechlorinating agent (e.g. sulfite or sulfur dioxide) is present, the sample result (corrected for the reagent blank) will read “0” or a slightly negative value.

13. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use.

Determining the reagent blank value



1. Select the test.

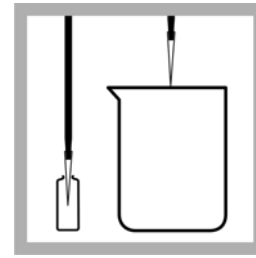
Make sure that the reagent blank setting is off.

See the user manual for information.

2. Install the Pour-Thru module.

Flush the Pour-Thru cell with 50 mL of deionized water.

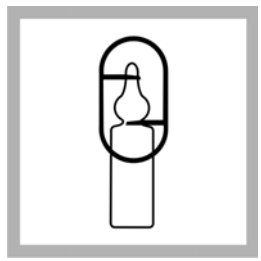
3. Collect about 100 mL of deionized or tap water in a clean, 250-mL beaker.



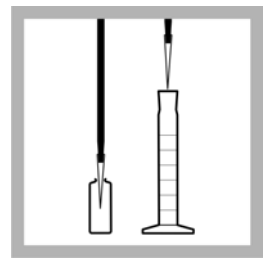
4. Use a TenSette® Pipet to add 1.0 mL of Blanking Reagent to the beaker. Swirl several times to mix.

The Blanking Reagent removes chlorine and chloramines from the water. ***Note: This solution will be used in Step 10.***

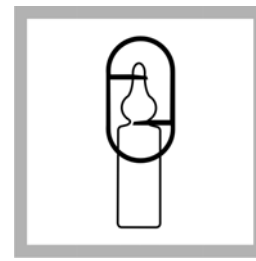
5. Access the general timer and set it for five minutes. Start the timer.



6. After the timer expires, open one ampule of ULR Chlorine Buffer Solution.

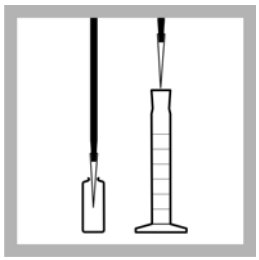


7. Use a TenSette Pipet and a clean tip to transfer 1.0 mL buffer from the ampule to a clean 50 mL mixing graduated cylinder.

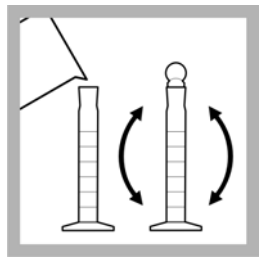


8. Open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine.

Determining the reagent blank value (continued)



9. Use a TenSette Pipet and a clean tip to transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix the reagents. Proceed to step 10 within one minute.

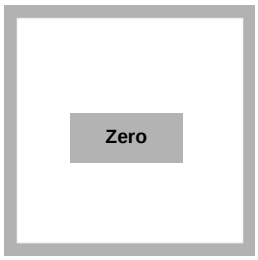


10. Fill the cylinder to the 50-mL mark with dechlorinated water from step 4. Cap and invert twice to mix. Save the remaining water for step 12.

11. Start the instrument timer.

A three-minute reaction time will begin.

12. During the reaction period, flush the Pour-Thru Cell with the remainder of original dechlorinated water from step 10.



13. When the flow stops, **ZERO** the instrument.

The display will show: 0 µg/L Cl_2.

14. When the timer expires, pour the contents of the cylinder into the Pour-Thru Cell. **READ** the results in µg/L chlorine.

15. Use this value to correct the sample result obtained in this procedure.

See the user manual for details on saving the reagent blank value.

16. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use.

Interferences

Table 117 Interfering substances and levels

Interfering substance	Interference levels and treatments
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Copper, Cu^{2+}	Greater than 1000 µg/L
Iodine, I_2	Interferes at all levels.
Iron (Fe^{3+})	Greater than 1000 µg/L

Table 117 Interfering substances and levels (continued)

<table>
<tr><th>Interfering substance</th><th>Interference levels and treatments</th></tr>
<tr><td>Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})</td><td>1. Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid[1].
2. Add 9 drops Potassium Iodide (30 g/L)[1] to an 80-mL sample.
3. Mix and wait 1 minute.
4. Add 9 drops Sodium Arsenite[1, 2] (5 g/L) and mix.
5. Analyze the treated sample as described in the procedure above.
6. Subtract the result of this test from the original analysis to obtain the correct concentration.</td></tr>
<tr><td>Nitrite, NO_2^- (uncommon in clean waters)</td><td><table><tr><th>mg/L nitrite</th><th>Apparent µg/L chlorine</th></tr><tr><td>2.0 mg/L</td><td>3 µg/L</td></tr><tr><td>5.0 mg/L</td><td>5 µg/L</td></tr><tr><td>10.0 mg/L</td><td>7 µg/L</td></tr><tr><td>15.0 mg/L</td><td>16 µg/L</td></tr><tr><td>20.0 mg/L</td><td>18 µg/L</td></tr></table></td></tr>
<tr><td>Ozone</td><td>Interferes at all levels</td></tr>
<tr><td>Peroxides</td><td>May interfere</td></tr>
<tr><td>Extreme sample pH or highly buffered samples</td><td>Adjust to pH 6–7</td></tr>
</table>

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). Refer to the current MSDS for safe handling and disposal instructions.

Sample collection, storage and preservation

- Analyze samples for chlorine immediately after collection. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- A common error in testing for chlorine is failure to obtain a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Treating analysis labware

Glassware used in this test must be chlorine demand-free. Fill the 100-mL mixing cylinder and sample container with a dilute solution of chlorine bleach prepared by adding 1 mL of commercial bleach to 1 liter of water. Soak in this solution at least one hour. After soaking, rinse thoroughly with deionized water and allow to dry before use.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes. Rinse several times with deionized water.

Cleaning the Pour-Thru cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N Sulfuric Acid followed by several rinsings with deionized water.

Accuracy check

Required for accuracy check:

- Low Range Chlorine PourRite® Ampule Standard Solution, 25 to 30-mg/L (25,000 to 30,000 µg/L) Cl_2
- TenSette® Pipet and tips
- Ampule Breaker

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Enter the chlorine concentration from the ampule package. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Low Range Chlorine Voluette Ampule Standard Solution, 25 to 30-mg/L (25,000 to 30,000 µg/L) Cl_2.
5. Prepare three sample spikes. Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 50-mL samples, respectively. Swirl gently to mix.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Point of curve	Concentration
86	295 µg/L Cl_2	290–300 µg/L Cl_2	Entire range	17 µg/L Cl_2

Summary of method

This method is designed for clean water, low in color and turbidity. The main applications include monitoring for trace chlorine break-through of activated carbon beds and feedwater to reverse osmosis membranes or ion-exchange resins.

Several modifications to the normal DPD chlorine method are necessary to measure trace levels of chlorine. The Pour-Thru Cell must be used in the spectrophotometer. Liquid reagents are also required. The reproducible optics of the Pour-Thru Cell give more stable readings than is possible with movable sample cells, resulting in more stable measurements.

The reagents are packaged in ampules and sealed under argon gas to ensure stability. Use of liquid reagents eliminates any slight turbidity that might be caused by using powdered reagents. Due to the possible oxidation of the reagents (which could give a positive chlorine reading in the blank), a reagent blank must be determined at least once a day for each lot of reagent used. This reagent blank value is subtracted from the sample result and the corrected value is the actual chlorine concentration. Test results are measured at 515 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Catalog number
ULR Chlorine Reagent Set (approximately 20 tests), includes:			2563000
ULR Chlorine Buffer Solution, 1.5-mL ampules	1 mL	20/pkg	2493120
DPD Indicator Solution for ULR Chlorine, 1.5-mL ampules	1 mL	20/pkg	2493220
Blanking Reagent for ULR Chlorine	1 mL	29 mL	2493023

Required Apparatus

Description	Quantity	Unit	Catalog number
PourRite® Ampule Breaker	1	each	2484600
Beaker, 250-mL	1	each	50046H
Cylinder, graduated mixing, 50-mL	1	each	189641
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	2	50/pkg	2185696

Recommended Standards

Description	Unit	Catalog number
Chlorine Standard Solution, PourRite® Ampule, 25–30 mg/L, 2-mL	20/pkg	2630020

Optional reagents and apparatus

Description	Unit	Catalog number
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sulfuric Acid, 1 N	100 mL	127032
Sulfuric Acid, 5.25 N	1000 mL	244953
pH Paper, 0 - 14 pH range	100/pkg	2601300
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Voluette Ampule breaker 10 mL	each	2196800
Chlorine Standard Solution, 2-mL PourRite® Ampules, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette® ampules, 50–75 mg/L	16/pkg	1426810

[1] other sizes are available

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Edition 7

Chlorine, Free and Total

DOC316.53.01172

DPD-FEAS Method **Method 8210**

0 to 3.00 mg/L Cl_2 **Digital Titrator**

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

It is best to use separate, dedicated flasks for free and total chlorine determinations.
mg/L combined chlorine = mg/L total chlorine – mg/L free chlorine
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

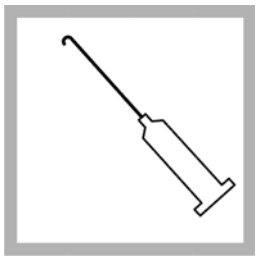
[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
DPD Free Chlorine Powder Pillow	1 pillow
DPD Total Chlorine Powder Pillow	1 pillow
Ferrous Ethylenediammonium Sulfate (FEAS) Titration Cartridge	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Pipet, volumetric, 25-mL, Class A and pipet filler	1
Erlenmeyer flask, 50-mL	1

See *Consumables and replacement items* for reorder information.

Chlorine, Free and Total



17. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

18. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

19. Use a pipet to measure 25.0 mL of the sample into a 50-mL Erlenmeyer flask.

20. Add the contents of one DPD Free Chlorine Powder Pillow. Swirl to mix.

Results will still be accurate if a small amount of powder does not dissolve.

21. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the solution is colorless.

Write down the number of digits displayed on the counter.

22. Calculate:

digits x 0.01 = mg/L Free Chlorine as Cl_2

Example: 25.0 mL of a sample was titrated and 250 digits were used to reach the endpoint. The concentration is 250 x 0.01 = 2.50 mg/L Cl_2

To measure total chlorine, follow the procedure with the following modification:

Substitute a DPD Total Chlorine Powder Pillow in step 20. Wait three minutes before proceeding to step 21. The result in step 22 will be expressed as mg/L total chlorine.

Accuracy check

Use the standard additions method to find if the sample has an interference and to confirm the analytical technique.

Standard additions method (sample spike)
Required for accuracy check:

- Chlorine Standard Solution, PourRite™ Ampule, 50–75 mg/L Cl_2 (the exact concentration will be shown on a certificate enclosed with the ampule)
- Ampule breaker

- TenSette Pipet, 0.1–1.0 mL

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 25-mL samples. Swirl to mix.
3. Follow the test procedure and titrate each of the spiked samples and a sample with no standard added.
4. Each 0.1 mL of standard that was added will use 20–30 digits to reach the endpoint. To find the exact number of digits that should be used for each 0.1-mL addition, multiply the exact concentration by 4 and by the spike volume.
   (Example: 50 mg/L x 0.1 mL x 4 = 20 digits)

   If more or less titrant was used, the problem can be due to user technique, an interference (see *Interferences*) or a problem with reagents or apparatus.

Interferences

Table 118 Interfering substances and levels

Interfering substance	Interference levels and treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested.
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30-g/L) to a 10-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops Sodium Arsenite [1] (5-g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or Highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid, 1.000 N) or base (Sodium Hydroxide, 1.00 N).
Temperature	Higher room temperatures tend to give higher free chlorine values due to reaction of chloramines. Higher room temperatures also result in increased color fading.

[1] Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See the current MSDS for proper disposal of hazardous material.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times,
- Cap the sample containers so there is no headspace (air) above the sample.
- Perform the chlorine analysis immediately.

Summary of method

The DPD-FEAS method provides a titrimetric procedure for determining free available chlorine and for estimating free and combined chlorine fractions that are present together. The magenta species, resulting from the oxidation of DPD by chlorine, is destroyed quantitatively by titration with ferrous ethylenediammonium sulfate. The volume of titrant required to reach a colorless end point is proportional to the chlorine concentration. Total residual chlorine may also be determined by this test.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Free and Total Chlorine Reagent Set (approximately 100 tests):			2445300
(1) DPD Free Chlorine Powder Pillows	1 pillow	100/pkg	1407099
(1) DPD Total Chlorine Powder Pillows	1 pillow	100/pkg	1406499
(1) FEAS Titration Cartridge, 0.00564 N	varies	each	2292301

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 50-mL	1	each	50541
Pipet, volumetric, Class A, 25-mL	1	each	1451540
Pipet Filler, safety bulb	1	each	1465100
Delivery tubes w/ 180° hook	1	each	1720500
Delivery tubes w/ 90° hook	1	each	4157800

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, PourRite™ Ampule, 50–75 mg/L Cl_2, 2-mL	20/pkg	1426820
PourRite Breaker	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Potassium Iodide Solution, 30-g/L	100 mL MDB	34332
Sodium Arsenite Solution, 5-g/L	100 mL MDB	104732
Sodium Hydroxide Standard Solution, 1.000 N	100 mL MDB	104532
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
Sulfuric Acid Standard Solution, 1.000 N	100 mL MDB	127032
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
pH Test Strip, 0–14 pH	100/pkg	2601300
Pipet tips	100/pkg	2185628
Pipet tips	50/pkg	2185696
Chlorine Standard, 50–75 mg/L, 10 mL	16/pkg	1426810
Chlorine Standard, 25–30 mg/L, 2 mL	16/pkg	2630020
Voluette breaker	each	2196800

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Edition 7

Chlorine, Free

DOC316.53.01220

Amperometric Forward Titration using 0.00564 N PAO[1]

Method 10024

(0 to 1000 µg/L Chlorine as Cl_2)

Digital Titrator

Scope and Application: For drinking water; USEPA Accepted for reporting

[1] Procedure is equivalent to Standard Method (18th ed.) 4500 Cl D for drinking water.

Test preparation

Before starting the test:

Make sure that the proper stir bar is used. The wrong size can cause the loss of chlorine, unstable readings and loss of method sensitivity, especially when measuring low level chlorine concentrations.
When a new probe is placed in service or when the probe has not been used recently, prepare it according to the Probe Stabilization instructions in the *Amperometric Titrator Instruction Manual*.

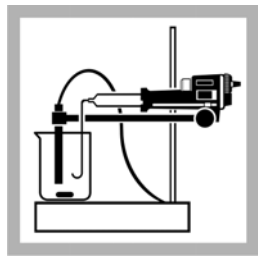
Collect the following items:

Description	Quantity
Amperometric Titrator assembly	each
Digital Titrator	each
Beaker, low-form, 250-mL	each
Stir bar, octagonal, Teflon-coated, 50.8 x 7.9 mm	each
Cylinder, graduated, 250-mL	each
TitraStir® mixer/stand assembly, 115 VAC	each
Probe Assembly, Amperometric Titrator	each
Delivery Tubes, 90° with hook	5/pkg
Phenylarsine Oxide Solution, 0.00564 N Digital Titrator Cartridge	each
Phosphate Buffer Solution, pH 7	1 bottle

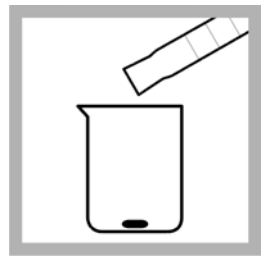
See *Consumables and replacement items* for reorder information.

Amperometric forward titration using 0.00564 N PAO

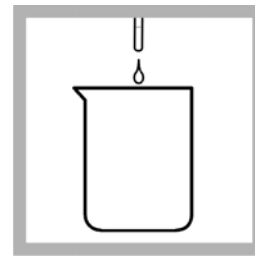
1. Install the 0.00564 N Phenylarsine Oxide (PAO) cartridge. Flush the Digital Titrator delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.



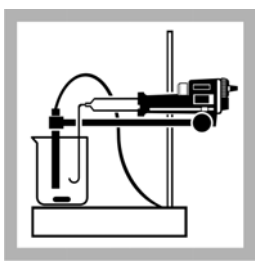
2. Assemble the Amperometric Digital Titrator System according to the instructions in the *Amperometric Titrator Instruction Manual*.



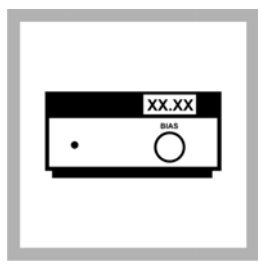
3. Without excessive agitation, measure 200 mL of sample with a clean graduated cylinder. Transfer the sample to a clean, 250-mL beaker containing the 50-mm stirring bar supplied with the system.



4. If the pH is less than 6 or greater than 7.5, add 1.0 mL of pH 7 Phosphate Buffer Solution to make the prepared sample.

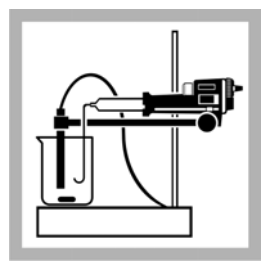


5. Place the beaker on the TitraStir® stand and immerse the tips of both the probe and the delivery tube in the solution. The probe's platinum wires must be submerged. Turn on the stirring motor.



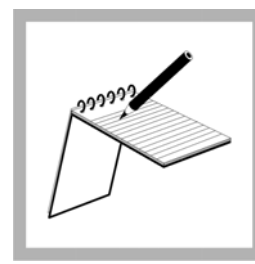
6. Note the LED reading on the Amperometric Titrator. Unlock the BIAS control and adjust the BIAS control knob until a reading between 0.50 and 0.06 is obtained. Lock the bias control.

The BIAS adjustment controls the slope of the titration curve. The actual instrument reading is not important; the relative readings as the titration proceeds are important. A precise adjustment is not required.



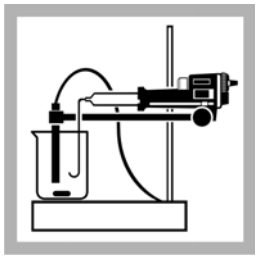
7. Using the Digital Titrator delivery knob, dispense the PAO Titrant Solution in 5 to 10 digit increments while noting the downward reading.

If the chlorine content of the sample is high, add titrant at a faster rate.

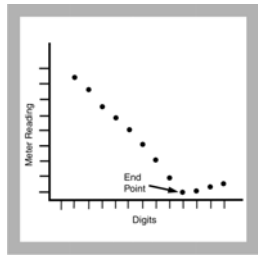


8. As the end point of the titration is approached, record the LED readings along with the corresponding digits displayed on the Digital Titrator counter. Near the titration end point, add 2 to 5 digits of titrant; wait a few seconds for a stable reading and record.

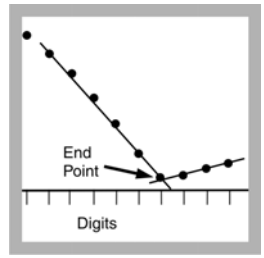
Amperometric forward titration using 0.00564 N PAO



9. Continue the titration, recording at least three points on the downward sloping curve and at least three points after the end point is reached. The latter points will cause little change in the LED readings.



10. Use linear graph paper to plot the recorded readings from the Amperometric Titrator on the vertical axis and the corresponding Digital Titrator digits on the horizontal axis.



11. Draw the two best intersecting lines through the points plotted as shown above. Determine the number of digits at the intersection of the two lines. This is the end point.

12. Determine the mg/L free chlorine concentration:

Digits at end point x 1.25 = µg/L free chlorine as Cl_2

Interferences

Table 119 Interfering substances

Interfering substance	Interference
Silver ions	Silver ions poison the electrode.
Copper ions	Interfere by plating on the electrode
High turbid water	Turbid water containing surface active agents
Oxidized manganese and other oxidizing reagents	Positive interference
Samples containing organic content	Some uncertainty in the endpoint may be observed on samples with high organic content.
Samples containing reducing agents	Excess reducing agents, such as sulfur dioxide, sulfite and bisulfite, will cause either static, or increasing LED readings because no free chlorine is present; these samples cannot be titrated under the conditions of the test.
Buffered samples or extreme sample pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the buffer reagent. If necessary, add additional buffer and check pH of sample prior to titration.

Sample collection, preservation and storage

Chlorine is rapidly lost from water.

- Avoid exposure to sunlight or other strong light.
- Avoid excessive agitation.
- Analyze samples immediately.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Chlorine Standard Solution Ampule
- TenSette® Pipet and tips
- Fresh sample

1. Snap the top off a Chlorine Standard Solution Ampule. Note the certificate concentration of the standard in mg/L.
2. Split a fresh sample into two 200-mL portions.
3. Use a TenSette® Pipet to add 0.1 to 0.5 mL of the standard to one portion and swirl to mix. This is the spiked sample.
4. Analyze both the sample and spiked sample and record the chlorine concentration of each.
5. Calculate the theoretical concentration of the spiked sample:

$$\text{Theoretical concentration} = \frac{(C_u \times V_u) + (C_s \times V_s)}{V_u + V_s}$$

Where:
C_u = measured concentration of sample, in mg/L (µg/L divided by 1000)

V_u = volume of sample

C_s = concentration of chlorine standard (mg/L, certificate value)

V_s = volume of standard added

6. Calculate the percent spiked recovery:

$$\text{\% Spike recovery} = \frac{\text{Spiked sample result, in mg/L}}{\text{Theoretical concentration calculated, in mg/L}}$$

Example:
Sample result (C_u) = 120 µg/L or 0.120 mg/L

Spiked sample result = 185 µg/L or 0.185 mg/L

Volume Sample (V_u) = 200 mL

Volume Standard (V_s) = 0.2 mL

Chlorine Standard (C_s) = 68.1 mg/L

$$\text{Theoretical concentration} = \frac{(0.120 \times 200) + (68.1 \times 0.2)}{200 + 0.2} = 0.188 \text{ mg/L}$$

$$\text{\% Spike recovery} = \frac{0.185 \text{ mg/L}}{0.188 \text{ mg/L}} \times 100 = 98\%$$

Ideally, the percent recovery should be 100%. Generally, results from 80–120% recovery are considered acceptable.

Method performance

Precision
In a single laboratory, a single operator used a standard solution of 338 µg/L chlorine to obtain a standard deviation of ±5.2 µg/L chlorine.

Detection Limit
With good operator technique, the estimated detectable concentration is approximately 15 µg/L chlorine using 0.00564 N PAO.

Summary of method

In the amperometric forward titration procedure for free chlorine, a small electrical current is applied across two identical platinum electrodes. No current can flow between the electrodes unless a substance that can be oxidized at the anode and a substance that can be reduced at the cathode are both present. In the case of the free chlorine titration with phenylarsine oxide (PAO), chlorine is reduced to chloride at the cathode due to the addition of PAO, and PAO is oxidized from the +3 oxidation state to the +5 oxidation state at the anode. Prior to the end point of the titration, both free chlorine and chloride are present in solution; allowing current to flow, even with a very small applied potential. At the end point, no free chlorine remains and the solution cannot conduct even if excess PAO titrant is added. The end point is defined when no change in current occurs, signaling all free chlorine has been reacted.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Phenylarsine Oxide Solution, 0.00564 N Digital Titrator Cartridge	each	199901
Phosphate Buffer Solution, pH 7	100 mL MDB	2155332

Required apparatus

Description	Unit	Catalog number
Amperometric Titrator Assembly	each	1929900
Digital Titrator	each	1690001
Beaker, low-form, 250-mL	each	50046H
Cylinder, graduated, 250-mL	each	50846
Delivery Tubes, 90° with hook	5/pkg	4157800
Probe Assembly, Amperometric Titrator	each	1939000
Stir Bar, octagonal, Teflon-coated, 50.8 x 7.9 mm	each	2095355
TitraStir® Mixer/Stand Assembly, 115 VAC OR TitraStir® Mixer/Stand Assembly, 230 VAC	each	1940000 1940010

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution Ampule, 50–75 mg/L	20/pkg	1426820
Water, demineralized, each	4 L	27256

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Chlorine, Hypochlorite

DOC316.53.01219

Iodometric Method[1]

Method 10100

HR (50 to 150 g/L) as Cl_2 (5 to 15%)

Digital Titrator

Scope and Application: For testing concentrated liquid bleach (sodium hypochlorite, soda bleach) used as a disinfectant in drinking water or wastewater treatment

[1] Adapted from ASTM method D2022.

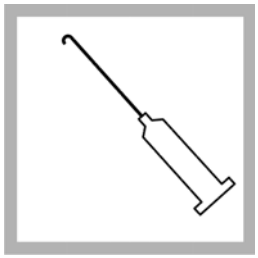
Test preparation

Collect the following items:

Description	Quantity
Digital Titrator Reagent Set, HR Hypochlorite (Bleach), 5–15% as Cl_2	1
Potassium Iodide-Iodate Standard Solution, 0.0125 N	1L
Clippers, large	1
Delivery Tubes, 180°	1
Digital Titrator	1
Flask, Erlenmeyer, 125-mL	1
TenSette Pipet, 0.1–1.0 mL	1
Tips, for TenSette® Pipet 1970001	1

See *Consumables and replacement items* for reorder information.

Iodometric method



13. Insert a clean delivery tube into the 2.26 N Sodium Thiosulfate Titrant Solution cartridge. Attach the cartridge to the titrator body.

14. Flush the delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe off the tip.

15. Fill the Erlenmeyer flask to about the 75-mL mark with deionized water or tap water.

The level of residual chlorine found in tap water will not interfere with the test.

16. Add the contents of one Potassium Iodide Powder Pillow to the flask and swirl to mix.

Iodometric method

17. Add the contents of one Acid Reagent Powder Pillow to the flask and swirl to mix.

18. Attach a clean tip to the TenSette Pipet.

Use the pipet to dispense 0.2 mL of bleach sample below the solution level in the flask.

19. Swirl to mix. The solution will turn dark brown.

20. Place the delivery tube tip into the solution and swirl the flask while titrating with the sodium thiosulfate titrant until the solution is pale yellow.

21. Add one dropperful of Starch Indicator Solution to the flask and swirl to mix. A dark blue or green color will develop.

22. Continue the titration until the solution becomes colorless. Record the number of digits required.

23. Calculate the g/L chlorine:

Digits required x 0.5 = g/L chlorine

g/L chlorine x 0.10 = % chlorine by volume (“trade percent”)

Interferences

The iodometric method is relatively free of interferences. The test will determine chlorite ion (ClO_2^-) in addition to the hypochlorite ion (ClO^-). However, the amount of chlorite in commercial bleach is insignificant (typically less than 0.2%).

Table 120 Interfering substances

Interfering substance	Interference level
Caustic agent	A large excess of caustic may cause low results. **1.** After adding the Acid Reagent Powder Pillow (step 17), check the pH of the solution with pH Paper. The pH should be less than 3. **2.** If the pH is not less than 3, add additional Acid Reagent, one pillow at a time, until the pH drops below 3.
Temperature	For most accurate results, the temperature of the dilution water should be less than 20 °C (68 °F).

Sample collection, preservation and storage

Soda bleach solutions are relatively unstable.

- Avoid exposing the sample to heat or light.
- Collect samples in glass bottles and store in a cool, dark place until analyzed.
- Analyze as soon as practical.

Accuracy check

Standard solution method
For optimum test results, the manufacturer strongly recommends that reagent accuracy be checked with each new lot of reagents. The strength of the Sodium Thiosulfate Standard Solution can be checked using Potassium Iodide-Iodate Standard Solution:

Required for accuracy check:

- Class A pipet, 50-mL
- Pipet filler
- 125-mL Erlenmeyer flask.
- Potassium Iodide Powder Pillow
- 50.00 mL of 0.0125 N Potassium Iodide-Iodate Standard Solution

7. Use a Class A pipet to transfer 50.00 mL of 0.0125 N Potassium Iodide-Iodate Standard Solution to a clean 125-mL Erlenmeyer flask.
8. Add the contents of one Potassium Iodide Powder Pillow to the flask and swirl to mix.
9. Add the contents of three Acid Reagent Powder Pillows to the flask and swirl to mix. Swirl until all powder is dissolved.
10. Continue the titration starting at step 20 of the procedure. It should take 217–227 digits of 2.26 N Thiosulfate Standard Solution to reach the end point.

Precision
In a single laboratory, using a commercial bleach sample of 91.2 g/L (9.12%) Cl_2, a single operator obtained a standard deviation of ±1.5 g/L (± 0.15%) Cl_2.

Summary of method

Under acidic conditions, hypochlorite reacts with iodide to produce an equivalent amount of triiodide (I_3^-). The released I_3^- is titrated with standard sodium thiosulfate solution to a colorless end point. The number of digits of sodium thiosulfate required is proportional to the hypochlorite concentration in the original bleach sample.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Digital Titrator Reagent Set, HR Hypochlorite (Bleach), 5–15% as Cl_2. Includes:			
Acid Reagent Powder Pillows	1 pillow	100/pkg	104299
Potassium Iodide Powder Pillows	1 pillow	50/pkg	2059996
Sodium Thiosulfate Standard Solution Cartridge, 2.26 N	varies	each	2686901
Starch Indicator Solution	1	100 mL MDB	34932

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Clippers, large	1	each	96800
Delivery Tubes, 180°	1	5/pkg	1720500
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, 125-mL	1	each	50543
TenSette Pipet, 0.1–1.0 mL	1	each	1970001
Tips, for TenSette® Pipet 1970001	1	50/pkg	2185696

Recommended standards

Description	Unit	Catalog number
Potassium Iodide-Iodate Standard Solution, 0.0125 N	1 L	1400153

Optional reagents and apparatus

Description	Unit	Catalog number
pH Test Strips, 0–14 pH	100/pkg	2601300
Bottle, sample, glass, 4 oz, with cap	3/pkg	2161303
Pipet, volumetric Class A, 50-mL	each	1451541
Pipet Filler, Safety Bulb	each	1465100

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Edition 7

Chlorine, Total

DOC316.53.01221

Amperometric Back Titration[1]

Method 10025

(6 to 1000 µg/L Chlorine as Cl_2)

Digital Titrator

Scope and Application: For drinking water and wastewater; USEPA accepted for reporting

[1] Procedure is equivalent to *Standard Method (18th ed.)* 4500-Cl C for wastewater.

Test preparation

Before starting the test

When a new probe is used or the probe has not been used recently, prepare it according to the Probe Stabilization instructions in the *Amperometric Titrator Instruction Manual*.
Use the proper stir bar (Catalog number 2095355). The wrong size can cause the loss of chlorine, unstable readings, and loss of method sensitivity, especially when measuring low level chlorine concentrations.
To preserve the strength of the iodine titrant solution, always remove the delivery tube from the Digital Titrator cartridge and replace the cap when not in use. Protect the iodine titrant solution from direct sunlight.
The sample may be fixed at the sample site for brief transportation delays— but not for sample storage. (This fixing technique is not acceptable for USEPA compliance monitoring.). See *Sample collection, preservation and storage* for more information.

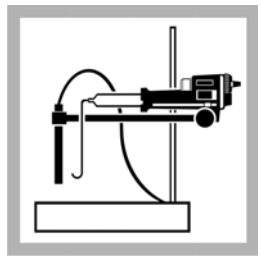
Collect the following items:

Description	Quantity
Standard Iodine Titrant Solution, Cartridge, 0.028 N	each
Digital Titrator	each
Cylinder, graduated, 250-mL	each
Amperometric Titrator Assembly	each
Beaker, low-form, 250-mL	each
Stir Bar, octagonal, Teflon-coated, 50.8 x 7.9 mm	each
Acetate Buffer Solution, pH 4.0	100 mL MDB
Potassium Iodide Powder Pillows	100/pkg
TitraStir® Mixer/Stand Assembly, 115 or 230 VAC	each
Pipet, Volumetric, Class A, 1-mL	each
Pipet Filler	each
Sodium Thiosulfate Standard Solution, 0.00564 N	100 mL
Probe Assembly, Amperometric Titrator	each
Delivery Tubes, 90° with hook	5/pkg

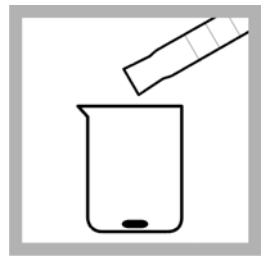
See *Consumables and replacement items* for reorder information.

Part 1—Adjusting the electrode response slope

1. Install the Standard Iodine Titrant Cartridge, 0.028 N. Flush the Digital Titrator delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

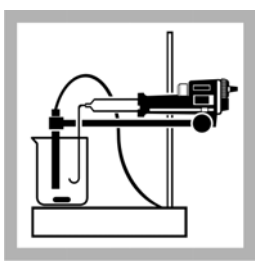


2. Assemble the Amperometric Digital Titrator System according to the instructions in the *Amperometric Titrator Instruction Manual*.

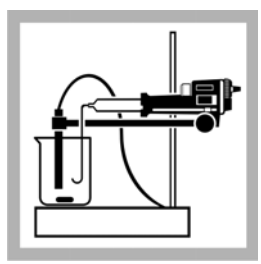


3. Use a graduated cylinder to measure 200 mL of deionized water into a clean 250-mL beaker. Place the 50-mm stirring bar into the beaker.

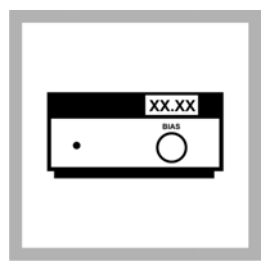
4. Add 1 mL of pH 4 Acetate Buffer and the contents of one Potassium Iodide Pillow.



5. Place the beaker on the TitraStir stand and immerse the tips of both the probe and the delivery tube in the solution. The probe's platinum wires must be submerged. Turn on the stirring motor.



6. Use the Digital Titrator delivery knob to add 50 digits of Standard Iodine Titrant Solution.



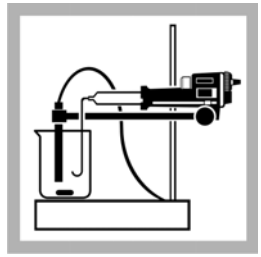
7. Note the LED reading on the Amperometric Titrator. Unlock the BIAS control knob until a stable reading between 0.50 and 0.60 is obtained. Lock the bias control.

8. Remove the probe arm from the beaker and rinse the platinum wires with deionized water. Discard the sample.

The adjustment of the electrode response slope is complete.

Part 2—Standardization of the Iodine Titrant

1. Flush the Digital Titrator delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.



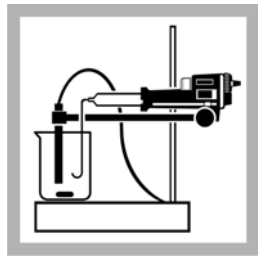
2. Assemble the Amperometric Digital Titrator System according to the instructions in the *Amperometric Titrator Instruction Manual*.

3. Use a graduated cylinder to measure 200 mL of deionized water into a clean 250-mL beaker. Place the 50-mm stirring bar into the beaker.

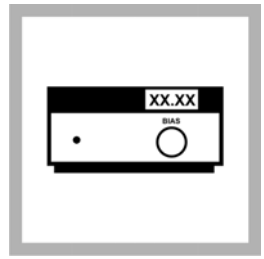
4. Use a Class A pipet to transfer 1.00 mL of 0.00564 N Sodium Thiosulfate Solution to the beaker. Swirl to mix.

Alternatively, use 0.00564 N Phenylarsine Oxide (PAO) (Catalog No. 199942) instead of sodium thiosulfate.

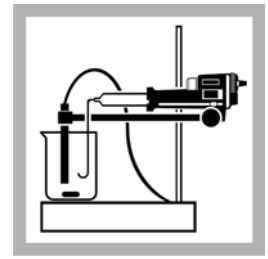
5. Add 1 mL of pH 4 Acetate Buffer Solution and the contents of one Potassium Iodide Powder Pillow.



6. Place the beaker on the TitraStir stand and immerse the tips of both the probe and the delivery tube in the solution. The probe's platinum wires must be submerged. Turn on the stirring motor.

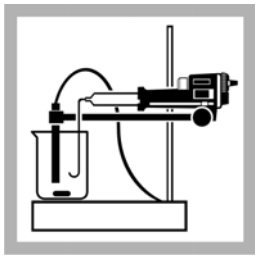


7. Note the LED reading on the Amperometric Titrator. It should read 0.00 ±0.05. **DO NOT** adjust the bias control.



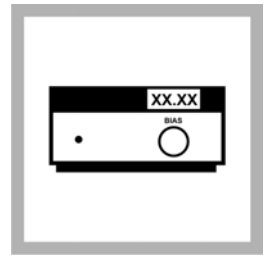
8. Using the Digital Titrator delivery knob, dispense 100 digits of Standard Iodine Titrant Solution and note the reading.

Part 2—Standardization of the Iodine Titrant (continued)

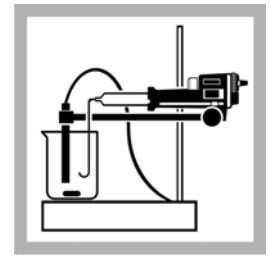


9. Continue dispensing titrant in five to ten digit increments while noting the reading.

10. Record at least three points (the null current values) before the end point is reached.

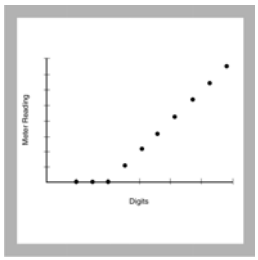


11. After the end point of the titration (nominal 160 digits), record the increasing LED readings along with the corresponding digits displayed on the Digital Titrator counter.

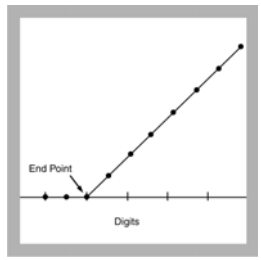


12. Add five to ten digits of titrant and wait a few seconds for a stable reading. Record it.

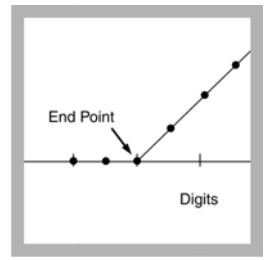
Stop adding titrant when the LED readings exceed 0.60. LED readings above 0.60 will be excessively noisy.



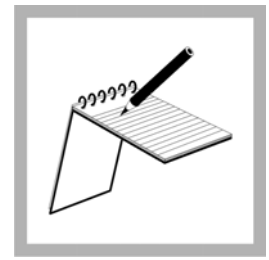
13. Use a linear graph paper to plot the recorded readings from the Amperometric Titrator on the vertical axis and the corresponding Digital Titrator digits on the horizontal axis.



14. Draw the two best intersecting lines through the plotted points as shown above.



15. Determine the number of digits at the intersection of the lines. That is the standard end point.



16. Record the standard end point digits value. Find the multiplier from the *Digit multipliers* table. This multiplier will be used to calculate the sample chlorine concentration.

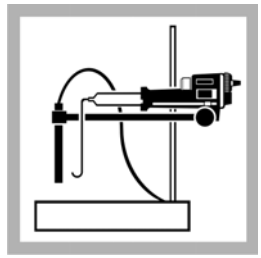
Discard the sample.

Table 121 Digit multipliers

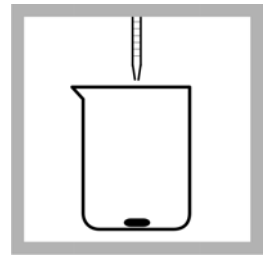
Digits (standard end point)	Multiplier
160	6.25
165	6.06
170	5.88
175	5.71
180	5.56
185	5.40
190	5.26
195	5.13
200	5.00

Part 3—Titration of sample for total residual Chlorine

1. Flush the Digital Titrator delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.



2. Assemble the Amperometric Digital Titrator System according to the instructions in the *Amperometric Titrator Instruction Manual*.



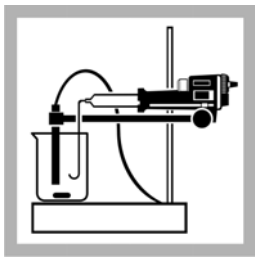
3. Place a clean, 50-mm stir bar into a clean 250-mL beaker. Use a Class A pipet to transfer 1.00 mL of 0.00564 N Sodium Thiosulfate Solution to the beaker.

Alternatively, use 0.00564 N Phenylarsine Oxide (PAO) (Catalog No. 199942) instead of sodium thiosulfate.

4. Add 1 mL of pH 4 Acetate Buffer Solution to the beaker. Then add 200 mL of the sample to the beaker.

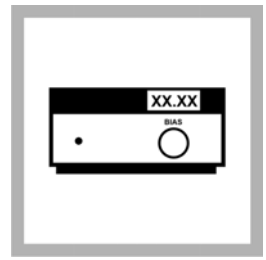
Minimize agitation when adding the sample.

Swirl to mix.

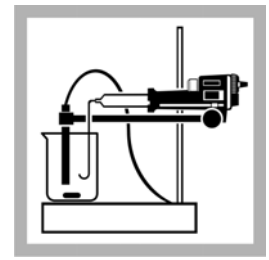


5. Place the beaker on the TitraStir stand and immerse the tips of both the probe and the delivery tube in the solution. The probe's platinum wires must be submerged. Turn on the stirring motor.

6. Add the contents of one Potassium Iodide Reagent Power Pillow to the beaker and allow the powder to dissolve.



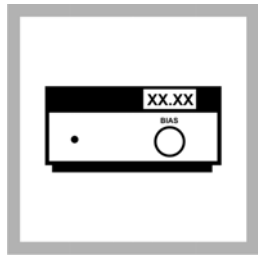
7. Note the LED reading on the Amperometric Titrator. It should read 0.00 ±0.05. **DO NOT** adjust the bias control.



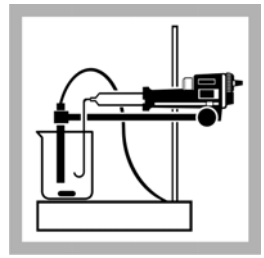
8. Using the Digital Titrator delivery knob, dispense Standard Iodine Titrant Solution in five to ten digit increments while noting the reading.

Part 3—Titration of sample for total residual Chlorine (continued)

9. Record at least three points (the null current values) before the end point is reached.

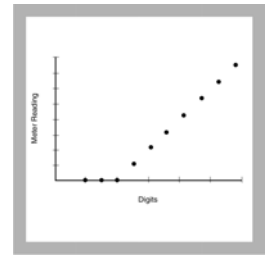


10. After the end point of the titration is reached, record the increasing LED readings along with the corresponding digits displayed on the Digital Titrator counter.

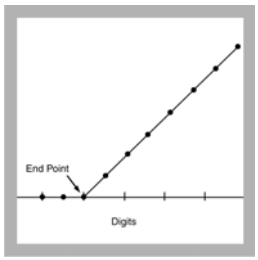


11. Add five to ten digits of titrant and wait a few seconds for a stable reading. Record it.

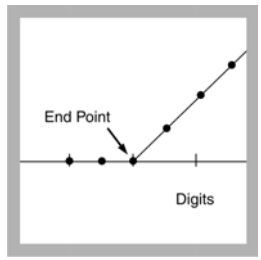
Stop adding titrant when the LED readings exceed 0.60. LED readings above 0.60 will be excessively noisy.



12. Using linear graph paper, plot the recorded readings from the Amperometric Titrator on the vertical axis and the corresponding Digital Titrator digits on the horizontal axis.



13. Draw the two best intersecting lines through the plotted points as shown above.



14. Determine the number of digits at the intersection of the lines. That is the sample end point.

15. Calculate the µg/L total chlorine:

[Digits (Standard End Point) – Digits (Sample End Point)] x Multiplier = µg/L Cl_2

Use the multiplier from *Part 2—Standardization of the Iodine Titrant*, step 16.

Interpolation between values in the table is not necessary.

Example:
Standard EP = 160 digits
Sample EP = 150 digits
Multiplier = 6.25, so:

µg/L total chlorine [160 – 150] x 6.25 = 62.5, or 63 (round up)

Table 122 Interfering substances

Interfering substance	Interference
Silver ions	Silver ions poison the electrode
Copper ions	Interfere by plating on the electrode.
Turbid water	Interferences are sometimes found in highly turbid water and those containing surface active agents
Oxidized manganese	Oxidized manganese and other oxidizing reagents give positive interferences.
Samples containing high organic content.	Some uncertainty in the end point may be observed with samples containing high organic content.
Iron and nitrite	Iron and nitrite interference are minimized by buffering to pH 4 before adding potassium iodide.
Buffered samples or sample pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the buffer reagent. If necessary, add additional buffer and check pH of sample prior to titration.
Dechlorinating agents	In samples that contain excess dechlorinating agents, such as sulfur dioxide, sulfite or bisulfite, the titration end point (number of digits) will be greater than the number of digits obtained during the standardization. It is not necessary to continue the titrant addition if the number of digits used in the sample titration exceeds that calculated for the standardization end point. This indicates that no free or combined chlorine is present in the sample.

Sample collection, preservation and storage

Chlorine is rapidly lost from water. Avoid exposure to sunlight or other strong light. Avoid excessive agitation. Analyze samples immediately or fix the sample by pre-addition of standard thiosulfate and buffer. To fix the sample:

1. Pipet 1.00 mL of 0.00564 N Sodium Thiosulfate and add 1.0 mL of Acetate Buffer into a clean, dry glass sampling bottle (e.g. BOD bottle).
2. At the sample site, measure 200 mL of sample with a graduated cylinder and transfer to the sampling bottle. Swirl to mix.
3. Before analysis, quantitatively transfer the entire contents of the sampling bottle to the 250-mL beaker. Minimize delay between sampling and analysis (1 hour maximum) to prevent decomposition of thiosulfate in the sample. (This fixing technique is not acceptable for USEPA compliance monitoring and should be used for brief transportation delays—not for sample storage.)
4. Start the analysis in *Part 3—Titration of sample for total residual Chlorine* at step 5.

Accuracy check

Use the bias control prior to performing the analysis to adjust the electrode sensitivity. Set the bias adjustment by adding a known amount of standard iodine titrant to deionized water and adjusting the bias control to a given value on the display. The electrode sensitivity will vary depending on the probe conditioning. Adjustment should be made at least daily or before each series of samples.

The iodine titrant concentration is approximately 0.0282 N, which relates to 160 digits needed to titrate 1.00 mL of 0.00564 N Thiosulfate. If the calculated end point is greater than 160 digits, this indicates that the Standard Iodine Titrant is weaker than when packaged. Discard the Standard Iodine Titrant cartridge if the calculated standard end point in *Part 2—Standardization of the Iodine Titrant* is greater than 200 digits.

To preserve the strength of the iodine titrant solution, always remove the delivery tube from the Digital Titrator cartridge and replace the cap when not in use. Protect the iodine titrant solution from direct sunlight.

Standard additions method (sample spike)

Note: *Standard additions is not applicable for samples containing excess reducing agents such as sulfur dioxide, sulfite, or bisulfite.*

Required for accuracy check:

- Chlorine Standard Solution Ampule
- TenSette Pipet and tips

1. Snap the top off a Chlorine Standard Solution Ampule. Note the certificate concentration of the standard in mg/L.

2. Split a fresh sample into two 200-mL portions.

3. Using a TenSette Pipet (Catalog number 1970001), add 0.1 to 0.5 mL of the standard to one portion and swirl to mix. This is the spiked sample.

4. Analyze both the sample and spiked sample and record the chlorine concentration of each.

5. Calculate the theoretical concentration of the spiked sample:

$$\text{Theoretical concentration} = \frac{(C_u \times V_u) + (C_s \times V_s)}{V_u + V_s}$$

Where:

C_u = measured concentration of sample, in mg/L (µg/L divided by 1000)

V_u = volume of sample

C_s = concentration of chlorine standard (mg/L, certificate value)

V_s = volume of standard added

6. Calculate the percent spiked recovery:

$$\text{\% Spike recovery} = \frac{\text{Spiked sample result, in mg/L}}{\text{Theoretical concentration calculated, in mg/L}}$$

Example:

Sample result (C_u) = 120 µg/L or 0.120 mg/L

Spiked sample result = 185 µg/L or 0.185 mg/L

Volume Sample (V_u) = 200 mL

Volume Standard (V_s) = 0.2 mL

Chlorine Standard (C_s) = 68.1 mg/L

$$\text{Theoretical concentration} = \frac{(0.120 \times 200) + (68.1 \times 0.2)}{200 + 0.2} = 0.188 \text{ mg/L}$$

$$\text{\% Spike recovery} = \frac{0.185 \text{ mg/L}}{0.188 \text{ mg/L}} \times 100 = 98\%$$

Ideally, the percent recovery should be 100%. Generally, results from 80–120% recovery are considered acceptable.

Method performance

Precision
In a single laboratory, using a standard solution of 120 µg/L chlorine, a single operator obtained a standard deviation of ±19 µg/L chlorine.

Detection limit
The estimated detectable concentration is equivalent to one digit of 0.0282 N Standard Iodine Titrant Solution, or approximately 6 µg/L chlorine.

Summary of method

The back titration procedure minimizes errors caused by liberating the full concentration of iodine in the sample and is the preferred method for amperometric measurement for total chlorine in wastewaters. In this procedure, the end point signal is reversed because the remaining thiosulfate (or phenylarsine oxide) added to the sample is titrated with standard iodine. The end point of the back titration is reached just when free iodine exists in the sample resulting in a measurable polarization current. The end point is estimated by continued addition of titrant, recording of the current at each titrant addition, and graphing the data points. Where the best line between the data points intersects the null current, the number of digits (from the Digital Titrator) at the end point can be determined and the chlorine concentration calculated.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Acetate buffer solution, pH 4.0	100 mL MDB	1490932
Potassium Iodide powder pillows	100/pkg	107799
Standard Iodine titrant solution, cartridge, 0.028 N	each	2333301
Sodium Thiosulfate standard solution, 0.00564 N	100 mL	2408842

Required apparatus

Description	Unit	Catalog number
Amperometric titrator assembly	each	1929900
Beaker, low-form, 250-mL	each	50046H
Cylinder, graduated, 250-mL	each	50846
Delivery tubes, 90° with hook	5/pkg	4157800
Digital titrator	each	1690001
Pipet, volumetric, Class A, 1-mL	each	1451535
Probe assembly, Amperometric titrator	each	1939000
Stir bar, octagonal, Teflon-coated, 50.8 x 7.9 mm	each	2095355
TitraStir® mixer/stand assembly, 115 VAC OR TitraStir® mixer/stand assembly, 230 VAC	each	1940000 1940010

Recommended standards

Description	Unit	Catalog number
Chlorine standard solution Ampule, 50–75 mg/L	20/pkg	1426820
Water, demineralized, each	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for 1970001	50/pkg	2185696

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Edition 7

Chlorine, Total

DOC316.53.01222

Amperometric Forward Titration[1]

Method 10026

(15 to 1000 µg/L Chlorine as Cl_2)

Digital Titrator

Scope and Application: For drinking water and wastewater; USEPA accepted for reporting

[1] Procedure is equivalent to *Standard Method (18th ed.)* 4500-Cl D for drinking water and *Standard Method (17th ed.)* 4500-Cl D for wastewater

Test preparation

Before starting the test:

When a new probe is used or the probe has not been used recently, prepare it according to the Probe Stabilization instructions in the *Amperometric Titrator Instruction Manual*.

Use the proper stir bar. The wrong size can cause the loss of chlorine, unstable readings and loss of method sensitivity, especially when measuring low level chlorine concentrations.

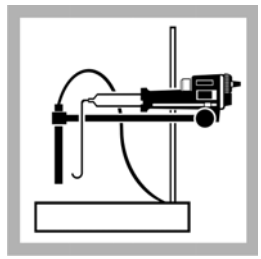
Collect the following items:

Description	Quantity
Phenylarsine Oxide Solution, 0.00564 N Digital Titrator Cartridge	each
Delivery Tubes, 90° with hook	5/pkg
Amperometric Titrator Assembly	each
Cylinder, graduated, 250-mL	each
Beaker, low-form, 250-mL	each
Stir Bar, octagonal, Teflon®-coated, 50.8 x 7.9 mm	each
Potassium Iodide Powder Pillows	100/pkg
Acetate Buffer Solution, pH 4	100 mL MBD
TitraStir® Mixer/Stand Assembly, 115 or 230 VAC	each
Digital Titrator	each
Graph paper	varies
Probe Assembly, Amperometric Titrator	each

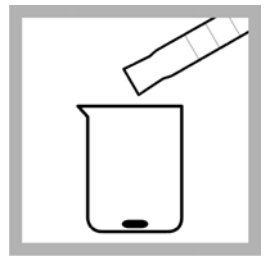
See *Consumables and replacement items* for reorder information.

Amperometric forward titration

1. Install the Phenylarsine Oxide (PAO) Cartridge, 0.00564 N. Flush the Digital Titrator delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.



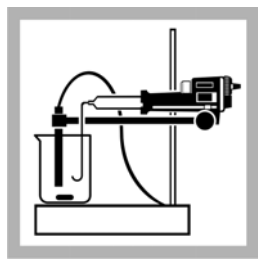
2. Assemble the Amperometric Digital Titrator System according to the instructions in the *Amperometric Titrator Instruction Manual*.



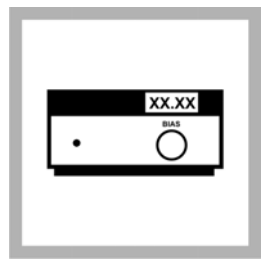
3. Without excessive agitation, measure 200 mL of sample with a clean graduated cylinder. Transfer the sample to a clean, 250-mL beaker containing the 50-mm stirring bar supplied with the system.

4. Add the contents of one Potassium Iodide Powder Pillow and swirl to dissolve.

5. Add 1 mL of pH 4 Acetate Buffer solution.

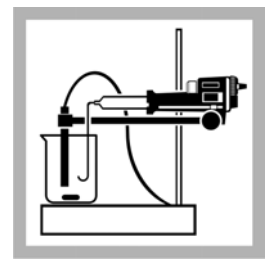


6. Place the beaker on the TitraStir stand and immerse the tips of both the probe and the delivery tube in the solution. The probe's platinum wires must be submerged. Turn on the stirring motor.



7. Note the LED reading on the Amperometric Titrator. Unlock the BIAS control and adjust the BIAS control knob until a stable reading between 0.50 and 0.60 is obtained. Lock the BIAS control.

The BIAS adjustment controls the slope of the titration curve. The actual instrument reading is not important; the relative readings as the titration proceeds are important. A precise adjustment is not required.

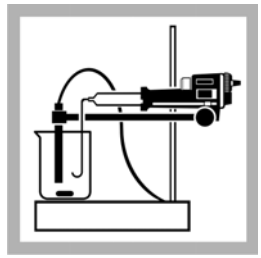


8. Using the Digital Titrator delivery knob, dispense the PAO Titrant Solution in 5 to 10 digit increments while noting the downward reading.

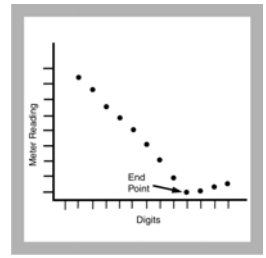
If the chlorine content of the sample is high, add titrant at a faster rate.

Amperometric forward titration (continued)

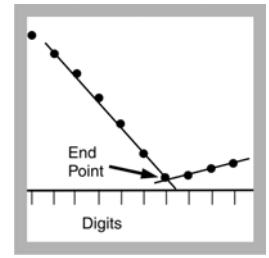
9. As the end point of the titration is approached, record the LED readings along with the corresponding digits displayed on the Digital Titrator counter. Near the titration end point, add 2 to 5 digits of titrant; wait a few seconds for a stable reading and record.



10. Continue the titration, recording at least three points on the downward sloping curve and at least three points after the end point is reached. The latter points will cause little change in the LED readings.



11. Using linear graph paper, plot the recorded readings from the Amperometric Titrator on the vertical axis and the corresponding Digital Titrator digits on the horizontal axis.



12. Draw the two best intersecting lines through the points plotted as shown above. Determine the number of digits at the intersection of the two lines. This is the end point.

13. Determine the µg/L total chlorine concentration:

Digits at end point x 1.25 = µg/L total chlorine as Cl_2

Interferences

Table 123 Interfering substances

Interfering substance	Interference
Silver ions	Silver ions poison the electrode.
Copper ions	Interfere by plating on the electrode.
Turbid water and water containing surface active agents	Interferences are sometimes found in highly turbid water and those containing surface active agents.
Oxidized manganese and other oxidizing reagents	Oxidized manganese and other oxidizing reagents give positive interferences.
Samples containing organic content.	Some uncertainty in the endpoint may be observed on samples with high organic content.
Samples containing excess reducing agents	Samples containing excess reducing agents, such as sulfur dioxide, sulfite and bisulfite do not contain free chlorine or chloramines and can not be titrated under the conditions of the test.
Buffered samples or sample pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the buffer reagent. If necessary, add additional buffer and check pH of sample prior to titration.

Sample collection, preservation and storage

Chlorine is rapidly lost from water.

- Avoid exposure to sunlight or other strong light.
- Avoid excessive agitation.
- Analyze samples immediately.

Accuracy check

Standard additions method* (sample spike)
Required for accuracy check:

- Chlorine Standard Solution Ampule, 50–75 mg/L Cl_2
- TenSette Pipet and tips

1. Snap the top off a Chlorine Standard Solution Ampule, 50–75 mg/L Cl_2. Note the specific certificate concentration of the standard in mg/L.
2. Split a fresh sample into two 200-mL portions.
3. Use the TenSette® Pipet (Catalog. Number. 1970001) to add 0.1 to 0.5 mL of the standard to one portion and swirl to mix. This is the spiked sample.
4. Analyze both the sample and spiked sample and record the chlorine concentration of each.
5. Calculate the theoretical concentration of the spiked sample:

$$\text{Theoretical concentration} = \frac{(C_u \times V_u) + (C_s \times V_s)}{V_u + V_s}$$

* The Standard Additions technique is not applicable to samples that contain excess reducing agents such as sulfur dioxide, sulfite, or bisulfite.

Where:
C_u = measured concentration of sample, in mg/L (µg/L divided by 1000)

V_u = volume of sample

C_s = concentration of chlorine standard (mg/L, certificate value)

V_s = volume of standard added

Calculate the percent spiked recovery:

$$\% \text{ Spike recovery} = \frac{\text{Spiked sample result, in mg/L}}{\text{Theoretical concentration calculated, in mg/L}}$$

Example:
Sample result (C_u) = 120 µg/L or 0.120 mg/L

Spiked sample result = 185 µg/L or 0.185 mg/L

Volume Sample (V_u) = 200 mL

Volume Standard (V_s) = 0.2 mL

Chlorine Standard (C_s) = 68.1 mg/L

$$\text{Theoretical concentration} = \frac{(0.120 \times 200) + (68.1 \times 0.2)}{200 + 0.2} = 0.188 \text{ mg/L}$$

$$\% \text{ Spike recovery} = \frac{0.185 \text{ mg/L}}{0.188 \text{ mg/L}} \times 100 = 98\%$$

Ideally, the percent recovery should be 100%. Generally, results from 80–120% recovery are considered acceptable.

Method performance

Precision
In a single laboratory, using a standard solution of 347 µg/L chlorine, a single operator obtained a standard deviation of ±3.2 µg/L chlorine.

Detection limit
The estimated detectable concentration is approximately 15 µg/L chlorine using 0.00564 N PAO.

Summary of method

In the amperometric forward titration procedure for total chlorine, a small electrical current is applied across two identical platinum electrodes. No current can flow between the electrodes unless a substance that can be oxidized at the anode and a substance that can be reduced at the cathode are both present. In the case of the total chlorine, an equivalent amount of iodine forms from the reaction of excess iodide with chlorine and combined chlorine at pH 4. During the titration with phenylarsine oxide (PAO), the free iodine is reduced to iodide at the cathode and PAO is oxidized from the +3 oxidation state to the +5 oxidation state at the anode. Prior to the end point of the titration, both iodine and iodide are present in solution; therefore current can flow, even with a very small applied potential. At the end point, no free iodine remains and the solution cannot conduct even if excess PAO titrant is added. The end point is defined when no change in current occurs, signaling all total chlorine has been reacted.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Phenylarsine Oxide Solution, 0.00564 N Digital Titrator cartridge	each	199901
Acetate Buffer Solution, pH 4	100 mL MBD	1490932
Potassium Iodide Powder Pillows	100/pkg	107799

Required apparatus

Description	Unit	Catalog number
Amperometric Titrator Assembly	each	1929900
Beaker, low-form, 250-mL	each	50046H
Cylinder, graduated, 250-mL	each	50846
Delivery Tubes, 90° with hook	5/pkg	4157800
Digital Titrator	each	1690001
Probe Assembly, Amperometric Titrator	each	1939000
Stir Bar, octagonal, Teflon®-coated, 50.8 x 7.9 mm	each	2095355
TitraStir® Mixer/Stand Assembly, 115 VAC OR TitraStir® Mixer/Stand Assembly, 230 VAC	each	1940000 1940010

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution Ampule, 50–75 mg/L	20/pkg	1426820
Water, deionized, each	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for 1970001	50/pkg	2185696

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Edition 7

Chlorine, Total

DOC316.53.01154

Iodometric Method using Sodium Thiosulfate[1]

Method 8161

0 to 40,000 mg/L

Buret Titration

Scope and Application: For water and seawater.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater (4500 Cl^- D).*

Test preparation

Before starting the test:

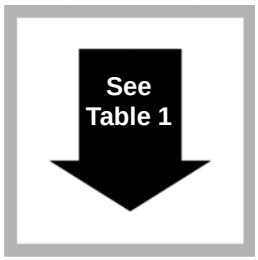
Chlorine can be lost from the sample during sample collection. Review the precautions in *Sample collection, preservation and storage* before the test is started.

Collect the following items:

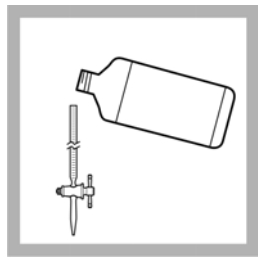
Description	Quantity
Dissolved Oxygen 3 Powder Pillow	1
Potassium Iodide Powder Pillow	1
Starch Indicator Solution	1 bottle
Sodium Thiosulfate Standard Solution (see the *Range-specific information* table)	1 bottle
Buret, Class A, 25-mL, with support stand	1
Erlenmeyer flask, 250 mL	1
Graduated cylinder	1

See *Consumables and replacement items* for reorder information.

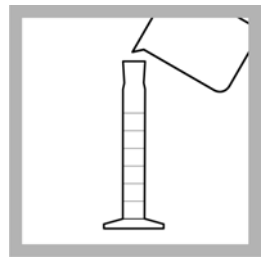
Buret titration



1. Select the sample volume and corresponding sodium thiosulfate solution from the *Range-specific information* table.



2. Fill a 25-mL buret to the zero mark with the Sodium Thiosulfate Standard Solution.



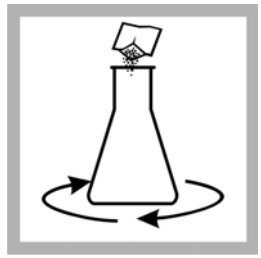
3. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table.

4. Transfer the sample into a 250-mL Erlenmeyer flask. If the sample volume is less than 50 mL, dilute to approximately 50 mL with deionized water.

Buret titration (continued)

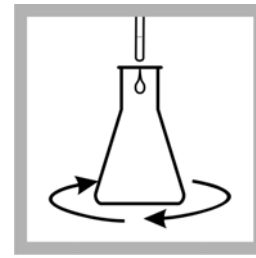
5. Add the contents of one Dissolved Oxygen 3 Powder Pillow. Swirl to mix.

The addition of the powder pillow will lower the pH to 4 or less. If the sample is highly alkaline, make sure that the solution pH is 4 or less with a pH meter or pH paper before proceeding.



6. Add the contents of one Potassium Iodide Powder Pillow. Swirl to mix.

7. Titrate the sample while swirling the flask until the color changes to a very light yellow.



8. Add one full dropper of Starch Indicator Solution. Swirl to mix. The sample will change to a dark blue color.

9. Titrate the sample while swirling the flask until the color changes from blue to colorless.

10. Calculate:

mL titrant used x multiplier = mg/L total chlorine as Cl_2

Example: 100 mL of sample was titrated with the 0.10 N sodium thiosulfate solution and 15 mL of titrant was used to reach the endpoint.
The chlorine concentration is: 15 x 35.5 = 532 mg/L Cl_2

Table 124 Range-specific information

Range (mg/L as Cl_2)	Sample volume (mL)	Sodium thiosulfate concentration	Multiplier
0–200	100	0.025 N	8.87
100–400	50	0.025 N	17.7
200–800	100	0.10 N	35.5
400–1,600	50	0.10 N	70.9
1,000–4,000	20	0.10 N	177
2,000–8,000	10	0.10 N	355
5,000–20,000	4	0.10 N	887
10,000–40,000	2	0.10 N	1773

Interferences

Oxidized forms of manganese, oxidizing agents and reducing agents such as organic sulfides can interfere.

Sample collection, preservation and storage

Start the chlorine analysis immediately after the samples are collected. Chlorine is a strong oxidizing agent and is not stable in natural waters. Chlorine reacts quickly with various inorganic compounds and slowly oxidizes organic compounds. Many factors such as sample composition, sunlight, pH, temperature and salinity can cause the decomposition of chlorine in water.

Do not use plastic containers because plastic can react with and consume chlorine. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of demineralized water) for at least 1 hour. Rinse thoroughly with demineralized or distilled water. If sample containers are rinsed thoroughly with demineralized or distilled water after use, only occasional pre-treatment is necessary.

Summary of method

When potassium iodide is added to a sample containing chlorine at a pH less than 4, free iodine is liberated in direct proportion to the amount of total chlorine present. The iodine is then titrated with sodium thiosulfate. Starch indicator is added to enhance the end point. This method measures both free chlorine and combined chlorine.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Dissolved Oxygen 3 Powder Pillows	1 pillow	100/pkg	98799
Potassium Iodide Powder Pillows	1 pillow	100/pkg	107799
Starch Indicator Solution	1 mL	100 mL MDB	34932
Titrant—select one or more based on range:			
Sodium Thiosulfate Standard Solution, 0.025 N	varies	1 L	35253
Sodium Thiosulfate Standard Solution, 0.10 N	varies	1 L	32353
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Clippers, for opening powder pillows	1	each	96800
pH Paper, 0–14 pH range	—	100/pkg	2601300
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 5-mL	1	each	50837
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Support Stand	1	each	56300

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Edition 7

Chlorine, Total

DOC316.53.01156

USEPA[1] Amperometric Buret Titration Method[2]

Method 8168

(0.5 mg/L and above)

Buret Titration

Scope and Application: For water and wastewater.

[1] USEPA accepted (4500 Cl^- D).

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

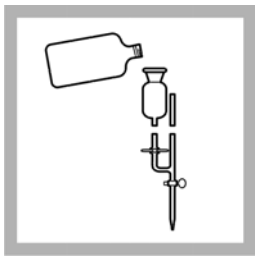
Chlorine can be lost from the sample during sample collection. Review the precautions in *Sample collection, preservation and storage* before the test is started.
Use only a 50-mm stir bar. The wrong size can cause the loss of chlorine, unstable readings and loss of method sensitivity, especially when measuring low level chlorine concentrations.
For added convenience when stirring, use the TitraStir® apparatus.
When a new probe is placed in service or when the probe has not been used recently, prepare it according to the Probe Stabilization instructions in the Amperometric Titrator Instruction Manual.

Collect the following items:

Description	Quantity
Phenylarsine Oxide Solution, 0.00564 N	1 bottle
Acetate Buffer Solution	1 mL
Potassium Iodide Powder Pillow	1
Beaker, 250-mL	1
Graduated cylinder, 250-mL	1

See *Consumables and replacement items* for reorder information.

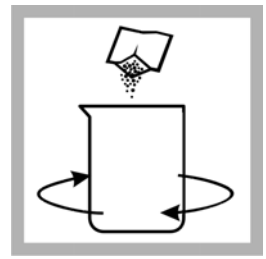
Buret titration



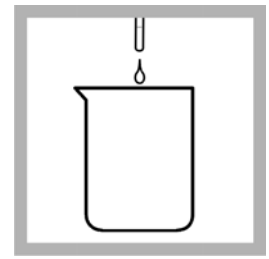
1. Fill the 5-mL automatic buret to the zero mark with 0.00564 N Phenylarsine Oxide (PAO) Solution.

2. Put a 50-mm stir bar into a 250-mL beaker.

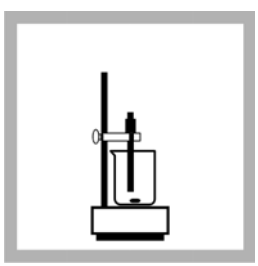
Use a graduated cylinder to measure 200 mL of sample. Add the sample to the beaker.



3. Add the contents of one Potassium Iodide Powder Pillow. Swirl to mix.

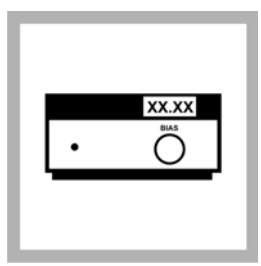


4. Add 1.0 mL of pH 4 Acetate Buffer Solution to make the prepared sample.



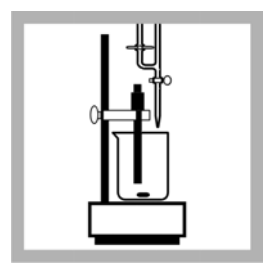
5. Place the beaker of prepared sample on the TitraStir titration stand and turn on the stirring motor. Put the tip of the probe fully into the prepared sample. The platinum wires must be submerged.

If a stir plate other than the TitraStir® is used, set the speed for moderate mixing. Do not adjust the speed after this point.

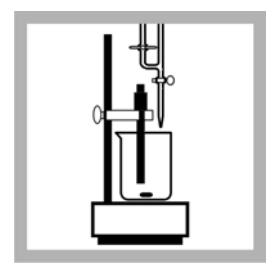


6. Turn the BIAS control knob to adjust the value on the display to approximately 1.00.

The BIAS adjustment controls the slope of the titration curve. The actual value is not important. Only the relative value as the titration continues is important. A precise adjustment is not necessary.

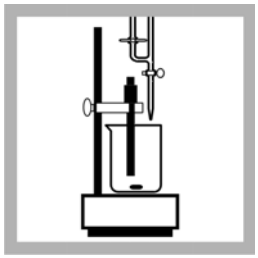


7. Dispense the titrant into the beaker in small increments while monitoring the values on the Amperometric Titrator. The values will decrease.

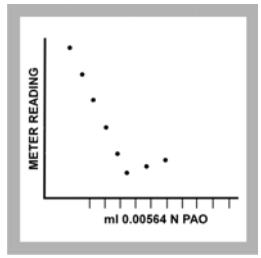


8. Continue dispensing slowly. Near the end point of the titration, write down the value on the display and the corresponding total volume of titrant that was added. Read the volume to the nearest 0.01 mL. Add a small amount of titrant and wait several seconds for a stable value. Write down the value.

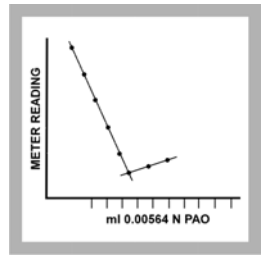
Buret titration (continued)



9. Continue the titration by recording at least three points on the downward sloping curve and at least three points after the end point has been reached. The value on the display will not change significantly after the end point.



10. Make a graph of the titration. Plot the values from the amperometric titrator on the vertical axis and the corresponding volume of titrant on the horizontal axis.



11. Draw the two best intersecting lines through the points as shown above. Find the volume of titrant to the nearest 0.01 mL at the intersection of the two lines. This is the mL titrant end point. This volume is equivalent to the total chlorine concentration in mg/L.

mL titrant =
mg/L total chlorine as Cl_2

Interferences

Refer to the Amperometric Titrator Instruction Manual for a discussion of sources of errors and interferences using the amperometric methods.

Sample collection, preservation and storage

Start the chlorine analysis immediately after the samples are collected. Chlorine is a strong oxidizing agent and is not stable in natural waters. Chlorine reacts quickly with various inorganic compounds and slowly oxidizes organic compounds. Many factors such as sample composition, sunlight, pH, temperature, and salinity can cause the decomposition of chlorine in water.

Do not use plastic containers because plastic can react with and consume chlorine. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of demineralized water) for at least 1 hour. Rinse thoroughly with demineralized or distilled water. If sample containers are rinsed thoroughly with demineralized or distilled water after use, only occasional pre-treatment is necessary.

A common error in testing for chlorine is introduced when a representative sample is not obtained. If sampling from a tap, let the water flow for at least 5 minutes before sample collection. Let the sample container overflow with the sample several times, then cap the container so that there is no headspace (air) above the sample. Start the chlorine analysis immediately.

Summary of method

Total chlorine is measured after the addition of potassium iodide and acetate buffer by a titration at pH 4 with PAO solution to the amperometric end point. The amperometric titration method has greater sensitivity and accuracy when compared to colorimetric methods. Refer to the Amperometric Titrator Instruction Manual for more information.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total Chlorine Reagent Set (approximately 200 tests), includes:			2460700
(2) Acetate Buffer Solution, pH 4	1 mL	100 mL MDB	1490932
(1) Phenylarsine Oxide Solution, 0.00564 N	varies	1 L	199953
(2) Potassium Iodide Powder Pillows	1	100/pkg	107799

Required apparatus

Description	Unit	Catalog number
Amperometric Buret Titrator System, 115 VAC	each	1930010
Amperometric Buret Titrator System, 230 VAC	each	1930012
Beaker, 250-mL	each	50046H
Graduated Cylinder, 250-mL	each	50846
Stir bar, 50 mm	each	2095355
TitraStir® apparatus, 115 VAC	each	1940000
TitraStir® apparatus, 230 VAC	each	1940010
pH paper, 0–14 pH range	100/pkg	2601300

Optional reagents and apparatus

Description	Unit	Catalog number
Chlorine Standard Solution, 10 mL Voluette® Ampules, 50–75 mg/L	16/pkg	1426810
Chlorine Standard Solution, 2 mL PourRite® Ampule, 50–75 mg/L	20/pkg	1426820
Voluette Ampule breaker, 10 mL	each	2196800
PourRite Ampule breaker, 2 mL	(each)	2484600
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L	20/pkg	2630020
Water, deionized	500 mL	27249

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Edition 7

Chlorine, Total

DOC316.53.01173

Iodometric Method Using Sodium Thiosulfate

Method 8209

1 to 400 mg/L or 20 to 70,000 mg/L Cl_2

Digital Titrator

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

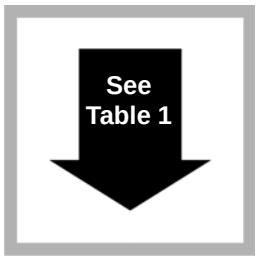
To convert mg/L chlorine to percent chlorine, divide the result in mg/L by 10,000.
These procedures can be used to measure iodine or bromine concentration if chlorine is not present. Multiply the test result (in mg/L chlorine) by 3.58 or 2.25, respectively, to accurately express the iodine or bromine content of the sample.
For added convenience when stirring, use the TitraStir® stirring apparatus.
For higher concentrations, see the hypochlorite procedure Method 10100.

Collect the following items:

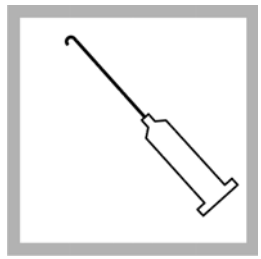
Description	Quantity
Acetate Buffer Solution, pH 4 (for 1 to 400 mg/L Cl_2 range)	1 bottle
Dissolved Oxygen 3 Powder Pillow (for 20 to 70,000 mg/L Cl_2 range)	1 pillow
Potassium Iodide Powder Pillow	1 pillow
Sodium Thiosulfate Ttitration cartridge (see *Range-specific information—20 to 70,000 mg/L*)	1 cartridge
Starch Indicator Solution	1 bottle
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 125-mL	1

See *Consumables and replacement items* for reorder information.

1 to 400 mg/L chlorine



1. Select a sample volume and titration cartridge from the *Range-specific information—1 to 400 mg/L* table.



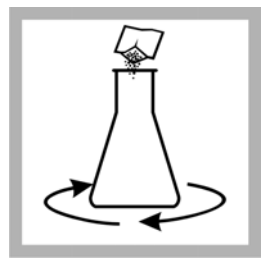
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

4. Use a clean graduated cylinder or pipet to measure the sample volume from the *Range-specific information—1 to 400 mg/L* table in a 125 mL Erlenmeyer flask.

5. Transfer the sample into a clean, 125-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.

6. Add two full droppers (2 mL) of Acetate Buffer Solution, pH 4. Swirl to mix.



7. Add the contents of one Potassium Iodide Powder Pillow. Swirl to mix.

8. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the solution is a pale yellow.

1 to 400 mg/L chlorine

9. Add one full dropper of Starch Indicator Solution. Swirl to mix.

A dark blue color will develop.

10. Continue the titration until the solution changes from dark blue to colorless.

Write down the number of digits displayed on the counter.

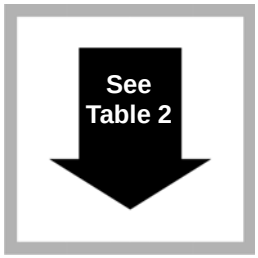
11. Use the multiplier in the *Range-specific information—1 to 400 mg/L* table to calculate the concentration:

digits x multiplier = mg/L Total Chlorine (Cl_2)
Example: 100 mL of sample was titrated and 250 digits were used to reach the endpoint. The concentration is 250 x 0.01 = 2.5 mg/L chlorine

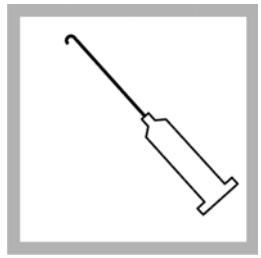
Table 125 Range-specific information—1 to 400 mg/L

Range (mg/L as Cl_2)	Sample volume (mL)	Titration cartridge (N)	Multiplier
1–4	100	0.02256	0.01
2–8	50	0.02256	0.02
5–20	20	0.02256	0.05
100–400	1	0.02256	1.0

20 to 70,000 mg/L chlorine



1. Select a sample volume and titration cartridge from the *Range-specific information—20 to 70,000 mg/L* table.

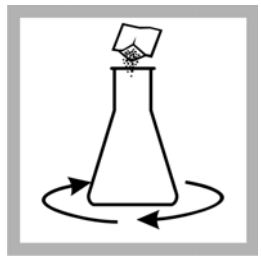


2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

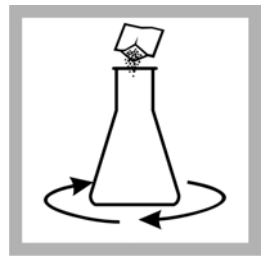
4. Use a clean graduated cylinder or pipet to measure the sample volume from the *Range-specific information—20 to 70,000 mg/L* table in a 125 mL Erlenmeyer flask.

5. Dilute to approximately 50 mL with deionized water.



6. Add the contents of one Dissolved Oxygen 3 Powder Pillow. Swirl to mix.

Normally, the addition of the powder pillow will lower the pH to 4 or less. If the sample is large or highly alkaline, make sure that the solution is pH 4 or less before proceeding.



7. Add the contents of one Potassium Iodide Powder Pillow. Swirl to mix.

8. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the solution is a pale yellow.

20 to 70,000 mg/L chlorine (continued)

9. Add one full dropper of Starch Indicator Solution. Swirl to mix.

A dark blue color will develop.

10. Continue the titration until the solution changes from dark blue to colorless.

Write down the number of digits displayed on the counter.

11. Use the multiplier in the *Range-specific information—20 to 70,000 mg/L* table to calculate the concentration:

digits x multiplier = mg/L Total Chlorine (Cl_2)

Example: 10 mL of sample was titrated with the 0.113 N titration cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.5 = 125 mg/L chlorine

Table 126 Range-specific information—20 to 70,000 mg/L

Range (mg/L as Cl_2)	Sample volume (mL)	Titration cartridge (N)	Multiplier
20–80	25	0.113	0.2
50–200	10	0.113	0.5
100–400	5	0.113	1.0
250–1000	2	0.113	2.5
500–2000	1	0.113	5
2000–9000 (0.2–0.9%)	4	2.00	22.2
5000–18,000 (0.5–1.8%)	2	2.00	44.3
10,000–35,000 (1.0–3.5%)	1	2.00	88.7
20,000–70,000 (2.0–7.0%)	0.5	2.00	177

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample.
- Perform the chlorine analysis immediately.

Accuracy check

Use the standard additions method to find if the sample has an interference and to confirm analytical technique.

Standard additions method (sample spike)
Required for accuracy check:

- Chlorine Standard Voluette® Ampule, 50–75 mg/L Cl_2 (the exact concentration will be shown on a certificate enclosed with the ampule)
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL

Procedure for use with the **0.02256 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively, to three samples of the same volume as used in the procedure. Swirl to mix.
3. Follow the test procedure and titrate each of the spiked samples and a sample with no standard added.
4. Each 0.2 mL of standard that was added will use approximately 10 digits to reach the endpoint. To find the exact number of digits that should be used for each 0.2-mL addition, multiply the exact concentration by the spike volume.
   (Example: 50 mg/L x 0.2 mL = 10 digits)

   If more or less titrant was used, the problem can be due to user technique, an interference or a problem with reagents or apparatus.

Procedure for use with the **0.113 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 1.0 mL, 2.0 mL and 3.0 mL of standard, respectively, to three samples of the same volume as used in the procedure. Swirl to mix.
3. Follow the test procedure and titrate each of the spiked samples and a sample with no standard added.
4. Each 1.0 mL of standard that was added will use approximately 10–15 digits to reach the endpoint. To find the exact number of digits that should be used for each 1.0-mL addition, multiply the exact concentration by the spike volume and divide by 5.
   (Example: 50 mg/L x 0.2 mL / 5 = 10 digits)

   If more or less titrant was used, the problem can be due to user technique, an interference or a problem with reagents or apparatus.

Summary of method

Total chlorine concentration equals the concentration of the free and the combined forms of chlorine. Free chlorine reacts readily with ammonia to form combined chlorine such as monochloramines. When potassium iodide is added to a sample containing chlorine at a pH less than 8, free iodine is liberated in direct proportion to the amount of total chlorine present. The iodine is then titrated with sodium thiosulfate.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
1–400 mg/L range:			
Acetate Buffer Solution, pH 4	2 mL	100 mL MDB	1490932
Potassium Iodide Powder Pillows	1 pillow	100/pkg	107799
Sodium Thiosulfate Titration Cartridge, 0.02256 N	varies	each	2409101
Starch Indicator Solution	1 mL	100 mL MDB	34932
20–2000 mg/L range—Reagent set (approximately 100 tests):			2272500
(1) Dissolved Oxygen 3 Powder Pillows	1 pillow	100/pkg	98799
(1) Potassium Iodide Powder Pillows	1 pillow	100/pkg	107799
(1) Sodium Thiosulfate Titration Cartridge, 0.113 N	varies	each	2267301
(1) Starch Indicator Solution	1 mL	100 mL MDB	34932
2000–70,000 mg/L range—Reagent set (approximately 100 tests):			2444800
(1) Dissolved Oxygen 3 Powder Pillows	1 pillow	100/pkg	98799
(2) Potassium Iodide Powder Pillows	1 pillow	50/pkg	2059996
(2) Sodium Thiosulfate Titration Cartridge, 2.00 N	varies	each	1440101
(1) Starch Indicator Solution	1 mL	100 mL MDB	34932

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator		each	1690001
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 5-mL	1	each	50837
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Delivery tubes w/ 180° hook	1	each	1720500
Delivery tubes w/ 90° hook	1	each	4157800

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Voluette® Ampule, 50–75 mg/L, 10-mL	16/pkg	1426810
Voluette Breaker	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TenSette Pipet, 1.0 to 10.0 mL	each	1970010
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
pH Test Strip, 0–14 pH	100/pkg	2601300
Pipet tips	100/pkg	2185628
Pipet tips	50/pkg	2185696
PourRite breaker	each	2484600

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Edition 7

Chromate

DOC316.53.01174

Titration Method using Sodium Thiosulfate **Method 8211**

20 to > 400 mg/L as CrO_4^{2-} **Digital Titrator**

Scope and Application: Closed system cooling towers.

Test preparation

Before starting the test:

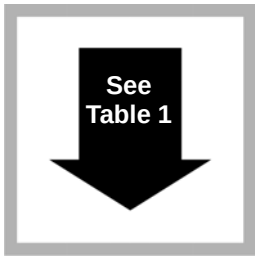
mg/L chromium = mg/L chromate x 0.448
mg/L sodium chromate = mg/L chromate x 1.4
For added convenience when stirring, use the TitraStir® stirring apparatus.

Collect the following items:

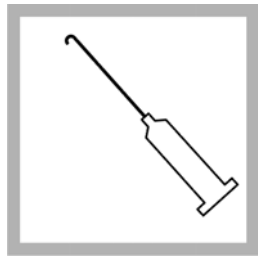
Description	Quantity
Potassium Iodide Powder Pillow	1 pillow
Dissolved Oxygen 3 Reagent Powder Pillow	1 pillow
Sodium Thiosulfate titration cartridge (see *Range-specific information*)	1 cartridge
Starch Indicator Solution	1 mL
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 125-mL	1

See *Consumables and replacement items* for reorder information.

Chromate



1. Select a sample volume and titration cartridge from the *Range-specific information* table.



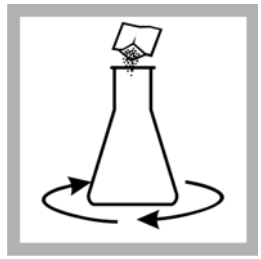
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

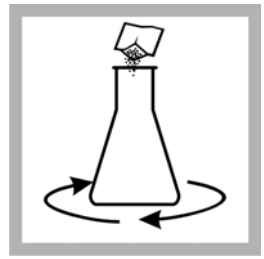
4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table in a 125 mL Erlenmeyer flask.

Chromate

5. Transfer the sample into a clean, 125-mL Erlenmeyer flask. If the sample volume is less than 50 mL, dilute to approximately 50 mL with deionized water.



6. Add the contents of one Potassium Iodide Powder Pillow. Swirl to mix.



7. Add the contents of one Dissolved Oxygen 3 Powder Pillow. Swirl to mix.

8. Wait 3 minutes.

Do not wait more than 10 minutes.

9. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the solution is a pale yellow.

10. Add one full dropper of Starch Indicator Solution. Swirl to mix.

A dark blue color will develop.

11. Continue the titration until the solution changes from dark blue to colorless.

Write down the number of digits displayed on the counter.

12. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L chromate (CrO_4^{2-})

Example: 50 mL of sample was titrated and 250 digits were used to reach the endpoint. The concentration is 250 x 0.2 = 50 mg/L CrO_4^{2-}

Table 127 Range-specific information

Range (mg/L as CrO_4^{2-})	Sample volume (mL)	Titration cartridge (N $Na_2S_2O_3$)	Multiplier
20–80	50	0.2068	0.2
50–200	20	0.2068	0.5
100–400	10	0.2068	1.0
> 400	5	0.2068	2.0

Interferences

Interfering substances lists substances that can interfere with this test.

Table 128 Interfering substances

Interfering substance	Interference level
Copper	Interfere to give high results. The effects of iron and copper can be masked by adding a Magnesium CDTA Powder Pillow, followed by two 1.0-gram measuring spoons of Sodium Acetate to the sample in step 7.
Iron, ferric (Fe^{3+})	
Other oxidants	Substances capable of oxidizing iodide to iodine under acidic conditions (such as ferric iron and copper) will interfere to give high results.

Sample collection, preservation and storage

- Collect samples in an acid-washed plastic or glass bottles.
- If sample can not be analyzed immediately, add 1 mL of concentrated sulfuric acid and swirl to mix.

Accuracy check

Use the standard additions method to determine whether the sample has an interference and to confirm the analytical technique.

Standard additions method (sample spike)
Required for accuracy check:

- Hexavalent Chromium Standard Solution, 1000-mg/L Cr^{6+}
- TenSette Pipet, 0.1–1.0 mL

5. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard to three samples. Use the same sample volume that was used for the analysis. Swirl to mix.
6. Follow the test procedure and titrate the spiked samples to the end point. Write down the amount of titrant that was used to reach the end point.
7. Each 0.1 mL of standard that was added will use approximately 22 digits of the titration cartridge to reach the endpoint.

   If more or less titrant was used, the problem can be due to user technique, an interference (see *Interferences*) or a problem with reagents or apparatus.

Standard solution method
Complete the following test to make sure that the reagents and user technique are accurate.

Required for accuracy check:

- Hexavalent Chromium Standard Solution, 1000-mg/L Cr^{6+}
- 100-mL volumetric flask, Class A

1. Use a pipet to add 3.0 mL of the standard solution, 1000-mg/L as Cr^{6+}, to a volumetric flask. Dilute to 100 mL with deionized water and mix fully. The diluted standard is equivalent to 67 mg/L chromate.
2. Use 20 mL or 50 mL of the standard as the sample volume and follow the test procedure.
3. Titrate the standard to the end point and calculate the result.

Summary of method

Chromate in the sample reacts with iodide under acidic conditions to form iodine as triiodide. The addition of starch indicator produces a blue color complex with the iodine. This complex is titrated with sodium thiosulfate to a colorless end point. The volume of titrant used is proportional to the chromate concentration.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chromate Reagent Set (approximately 100 tests):			2272400
(1) Dissolved Oxygen 3 Reagent Powder Pillows	1 pillow	100/pkg	98799
(2) Potassium Iodide Powder Pillows	1 pillow	50/pkg	2059996
(1) Sodium Thiosulfate Titration Cartridge, 0.2068 N	varies	each	2267601
(1) Starch Indicator Solution	1 mL	each	34932

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Delivery tubes w/ 180° hook	1	each	1720500
Delivery tubes w/ 90° hook	1	each	4157800

Recommended standards

Description	Unit	Catalog number
Chromium, Hexavalent, Standard Solution, 1000-mg/L as Cr^{6+}	100 mL	1466442

Optional reagents and apparatus

Description	Unit	Catalog number
Magnesium CDTA Powder Pillows	100/pkg	1408099
Sodium Acetate, trihydrate, ACS	454 g	17801H
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Bottle, sampling	250 mL	2087076
Sulfuric Acid, ACS	500 mL	97949
Measuring spoon	1 g	51000
Chromium standard, 50 mg/L	100 mL	81042H
Pipet tips	100/pkg	2185628
Pipet tips	50/pkg	2185696
Volumetric flask	100 mL	1457442
Volumetric Pipet	3 mL	1457442
Safety bulb	each	1465100
Tensette Pipet	1–10 mL	1970010
Clippers	each	96800

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Edition 7

Chromium, Hexavalent

DOC316.53.01033

USEPA[1] 1,5-Diphenylcarbohydrazide Method[2]

Method 8023

(0.010 to 0.700 mg/L Cr^{6+})

Powder Pillows or AccuVac® Ampuls

Scope and Application: For water and wastewater; USEPA accepted for reporting for wastewater analysis.[3]

[1] Accepted USEPA and Standard Method 3500 Cr B.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

[3] Procedure is equivalent to USGS method 1-1230-85 for wastewater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 129 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.
At high chromium levels, a precipitate will form. Sample dilution may be necessary.
The final samples are highly acidic. Refer to reagent MSDS sheets for disposal information.

Collect the following items:

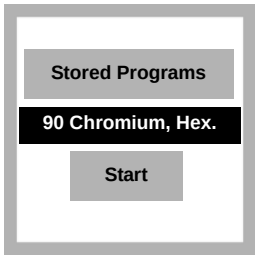
Description	Quantity
Powder Pillow Test:	
ChromaVer® 3 Chromium Reagent Powder Pillows	1
Sample cells, 10-mL	2
AccuVac Test:	
ChromaVer® 3 AccuVac® Ampuls	1
Beaker, 50-mL	1

Collect the following items:

Description	Quantity
Sample cell, 10-mL round, with cap	1

See *Consumables and replacement items* for reorder information.

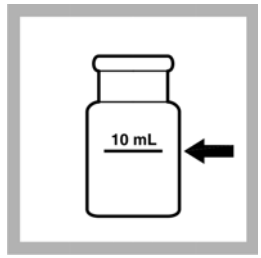
1,5-Diphenylcarbohydrazide for powder pillows



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



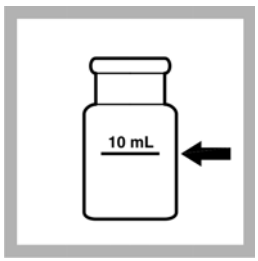
2. Fill a sample cell with 10 mL of sample.

3. Prepared Sample: Add the contents of one ChromaVer® 3 Reagent Powder Pillow to the sample cell. Swirl to mix.

A purple color will form if hexavalent chromium is present.

4. Start the instrument timer.

A five-minute reaction period will begin.



5. Blank Preparation: Fill a sample cell with 10 mL of sample.

6. When the timer expires, insert the blank into the cell holder.

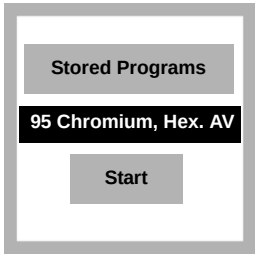
ZERO the instrument. The display will show:

0.000 mg/L Cr^{6+}

7. Insert the prepared sample into the cell holder.

READ the results in mg/L Cr^{6+}.

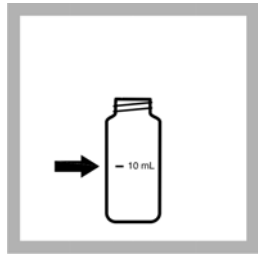
1,5-Diphenylcarbohydrazide for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a round sample cell with 10 mL of sample.

3. Prepared Sample: Fill a ChromaVer 3 Reagent AccuVac® Ampul with sample from the beaker. Keep the tip immersed while the Ampul fills completely.

4. Quickly invert the Ampul several times to mix.

5. Start the instrument timer.

A five-minute reaction period will begin.

6. When the timer expires, wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show:

0.000 mg/L Cr^{6+}

7. Wipe the Ampul and insert it into the cell holder.

READ the results in mg/L Cr^{6+}.

Interferences

Table 130 Interfering substances and levels

Interfering substance	Interference levels and treatments
Iron	May interfere above 1 mg/L
Mercurous & Mercuric Ions	Interfere slightly
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Vanadium	May interfere above 1 mg/L. Allow 10 minutes for the reaction period before reading.
Turbidity	For turbid samples, treat the blank with the contents of one Acid Reagent Powder Pillow[1]. This will ensure that any turbidity dissolved by the acid in the ChromaVer 3 Chromium Reagent will also be dissolved in the blank.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

Collect samples in a cleaned glass or plastic container. Store at 4 °C (39 °F) up to 24 hours. Samples must be analyzed within 24 hours.

Accuracy check

Required for accuracy check:

- Chromium Voluette® Ampule Standard, 12.5 mg/L Cr^{6+}
- TenSette Pipet and Pipet Tips
- Ampule Breaker

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Chromium Voluette® Ampule Standard, 12.5 mg/L Cr^{6+}.
5. For analysis using powder pillows, use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix thoroughly. Transfer 10 mL of each solution into a 10-mL sample cell and analyze as described above.

Note: *For AccuVac Ampuls, fill three mixing cylinders* with 50-mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers. Analyze each standard addition sample as described in the procedure above.*

6. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view relationships between the sample spikes and the “Ideal Line” of 100% recovery.

Standard solution method

Prepare a 0.50-mg/L Cr^{6+} standard solution daily, as follows:

1. Using a 5.00 mL pipet transfer 5.00 mL of Hexavalent Chromium Standard Solution, 50 mg/L, into a Class A 500-mL volumetric flask.
2. Dilute to the mark with deionized water. Perform the test procedure as described above.
3. To adjust the calibration curve using the reading obtained with the 0.50-mg/L Standard Solution, select **Options>More>Standard Adjust** from the instrument menu.

Note: *Refer to the instrument user manual for specific software navigation instructions.*

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

* See *Optional reagents and apparatus*.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
90	0.500 mg/L Cr^{6+}	0.497–0.503 mg/L Cr^{6+}	0.005 mg/L Cr^{6+}
95	0.500 mg/L Cr^{6+}	0.496–0.504 mg/L Cr^{6+}	0.006 mg/L Cr^{6+}

Summary of method

Hexavalent chromium is determined by the 1,5-Diphenylcarbohydrazide method using a single dry powder formulation called ChromaVer 3 Chromium Reagent. This reagent contains an acidic buffer combined with 1,5-Diphenylcarbohydrazide, which reacts to give a purple color when hexavalent chromium is present. Test results are measured at 540 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
ChromaVer® 3 Chromium Reagent Powder Pillows	1	100/pkg	1271099
OR			
ChromaVer® 3 AccuVac® Ampuls	1	25/pkg	2505025
Deionized Water	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Stopper for 18 mm Tube	1	6/pkg	173106
AccuVac Snapper	1	each	2405200

Recommended standards

Description	Unit	Catalog number
Chromium, Hexavalent Standard Solution, 10-mL Voluette® Ampules, 12.5-mg/L Cr^{6+}	16/pkg	1425610
Chromium, Hexavalent Standard Solution, 50.0-mg/L Cr^{6+}	100 mL	81042H

Optional reagents and apparatus

Description	Unit	Catalog number
Acid Reagent Powder Pillow	100/pkg	212699
Ampule Breaker	each	2196800
Flask, volumetric, Class A, 500-mL	each	1457449
Pipet, Volumetric 5.00 mL	each	1451537
Pipet FIller, safety bulb	each	1465100
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	245026

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Edition 7

Chromium, Total

DOC316.53.01034

Alkaline Hypobromite Oxidation Method[1, 2] **Method 8024**

0.01 to 0.70 mg/L Cr **Powder Pillows**

Scope and Application: For water and wastewater.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

[2] This procedure is equivalent to Standard Method 3500-Cr D for wastewater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 131 Instrument-specific information

Instrument	Sample cell	Orientation
DR 6000	2495402 (2) and 2401906 (1)	Fill line faces right
DR 5000	2495402 (2) and 2401906 (1)	Fill line faces user
DR 3900	2495402 (2) and 2401906 (1)	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402 (2) and 2401906 (1)	Fill line faces right

Before starting the test:

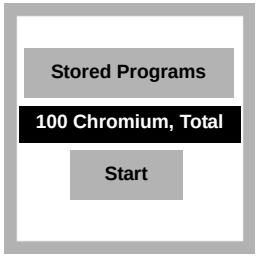
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Prepare a boiling water bath for step 4. Use finger cots to handle hot sample cells.

Collect the following items:

Description	Quantity
Acid Reagent Powder Pillows	1
ChromaVer® 3 Chromium Reagent Powder Pillows	1
Chromium 1 Reagent Powder Pillows	1
Chromium 2 Reagent Powder Pillows	1
Hot Plate	1
Water Bath and Rack	1
Finger Cots	1 pair
Sample Cells (*Instrument-specific information*)	varies

See *Consumables and replacement items* for reorder information.

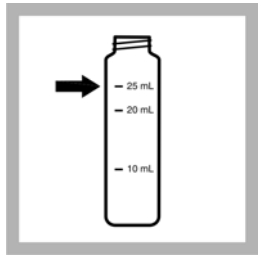
Alkaline Hypobromite Oxidation method for powder pillows



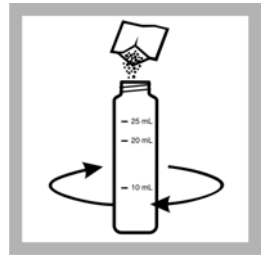
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a 25-mL sample cell with 25 mL of sample.



3. Prepared Sample: Add the contents of one Chromium 1 Reagent Powder Pillow.

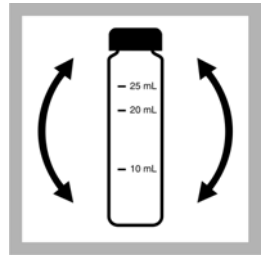
Swirl to mix.

4. Remove the cap. Insert the prepared sample into a boiling water bath.

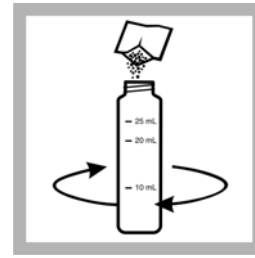
5. Start the instrument timer.

A five-minute reaction period will begin.

6. When the timer expires, remove the prepared sample. Cap the cell and use running water to cool the cell to 25 °C.



7. Remove cap and add the contents of one Chromium 2 Reagent Powder Pillow. Cap and invert to mix.



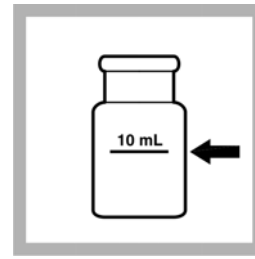
8. Add the contents of one Acid Reagent Powder Pillow. Swirl to mix.

9. Add the contents of one ChromaVer 3 Chromium Reagent Powder Pillow. Swirl to mix.

10. Start the instrument timer.

A five-minute reaction period will begin.

11. While the sample is reacting, pour 10 mL from the mixing bottle into a second sample cell (*Instrument-specific information*). This is the prepared sample.

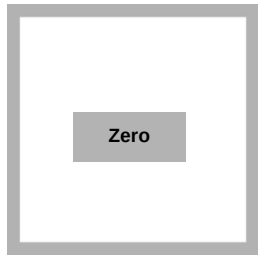


12. Blank Preparation:

When the timer expires, fill another sample cell with 10 mL of original sample.

Alkaline Hypobromite Oxidation method for powder pillows (continued)

13. Wipe the blank and insert it into the cell holder.

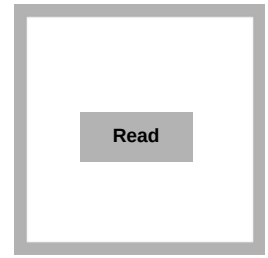


14. **ZERO** the instrument.

The display will show:

0.00 mg/L Cr

15. Wipe the prepared sample and insert it into the cell holder.



16. **READ** the results in mg/L Cr.

Interferences

Table 132 Interfering substances and levels

Interfering substance	Interference levels and treatments
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.
Organic material	May inhibit complete oxidation of trivalent chromium. If high levels of organic material are present, digestion may be required. Perform the analysis as described in this procedure on the digested sample.
Turbidity	For turbid samples, treat a 25-mL blank and the sample the same during steps 3–8. Use this solution as the blank.

Sample collection, preservation and storage

- Collect samples in acid-washed glass or plastic containers.
- To preserve samples, adjust the pH to 2 or less with nitric acid. This requires approximately 2 mL per liter of the acid.
- Store preserved samples at room temperature up to six months.
- Adjust the pH to about 4 with 5.0 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

Required for accuracy check:

- Trivalent Chromium Voluette® Ampule Standard, 50-mg/L as Cr^{3+}.
- TenSette Pipet and Pipet Tips
- Ampule Breaker
- Mixing cylinders

Standard additions method (sample spike)

5. Prepare a 12.5 mg/L standard by pipetting 5.00 mL of Trivalent Chromium Standard Solution, 50 mg/L as Cr3+ into a mixing cylinder. Pipet 15.00 mL of demineralized water into the cylinder. Stopper the cylinder and mix thoroughly.

6. After reading test results, leave the sample cell (unspiked sample) in the instrument.
7. Select Options>More>Standard Additions from the instrument menu.
8. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
9. Prepare three sample spikes. Fill three mixing cylinders with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
10. Analyze each sample spike as described in the procedure above, starting with the smallest sample spike. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.
11. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view relationships between the sample spikes and the "Ideal Line" of 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Prepare a 0.50-mg/L trivalent chromium standard as follows:

1. Dilute 5.00 mL of Trivalent Chromium Standard Solution, 50-mg/L as Cr^{3+}, to 500 mL with deionized water. Prepare this solution daily.
2. To adjust the calibration curve using the reading obtained with the 0.50-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
90	0.500 mg/L Cr	0.47–0.53 mg/L Cr	0.005 mg/L Cr

Summary of method

Trivalent chromium in the sample is oxidized to the hexavalent form by hypobromite ion under alkaline conditions. The sample is acidified. The total chromium content is determined by the 1,5-Diphenylcarbohydrazide method. Determine trivalent chromium by subtracting the results of a separate hexavalent chromium test from the results of the total chromium test. Test results are measured at 540 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total Chromium Reagent Set (100 tests), includes:	—	—	2242500
Acid Reagent Powder Pillows	1	100/pkg	212699
ChromaVer® 3 Chromium Reagent Powder Pillows	1	100/pkg	1206699
Chromium 1 Reagent Powder Pillows	1	100/pkg	204399
Chromium 2 Reagent Powder Pillows	1	100/pkg	204499

Required apparatus

Description	Quantity	Unit	Catalog number
Hot plate, 3½-inch diameter, 120 VAC, 50/60 Hz	1	each	1206701
OR			
Hot Plate, stirrer, 220 – 240 VAC	1	each	2881602
Water Bath and Rack	1	each	195555

Recommended standards

Description	Unit	Catalog number
Chromium, Trivalent, Standard Solution, 50-mg/L Cr^{3+}	100 mL	1415142

Optional reagents and apparatus

Description	Unit	Catalog number
Finger Cots	2/pkg	1464702
Flask, volumetric, Class A, 500 mL	each	1457449
Pipette, volumetric, Class A, 5.00 mL	each	1451537
Tensette Pipet 0.1–1.0 mL	each	1970001
Pipet Tips, Tensette	50/pkg	2185696
Pipet, Volumetric Class A, 15 mL	each	1451539
Pipet Filler, Safety Bulb	each	1465100
Cylinder, Mixing, 25 mL	each	189640

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Edition 7

Cobalt

DOC316.53.01036

1-(2-Pyridylazo)-2-Naphthol (PAN) Method[1] **Method 8078**

0.01 to 2.00 mg/L **Powder Pillows**

Scope and Application: For water and wastewater; digestion is required for determining total recoverable cobalt; if EDTA is present, use the vigorous digestion.

[1] Adapted from Watanbe, H., Talanta, 21 295 (1974).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 133 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

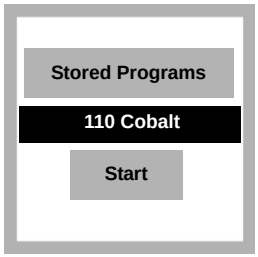
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
If the sample is less than 10 °C (50 °F), warm to room temperature prior to analysis.
Nickel can be determined with the same sample prepared with this method. Use program Number 340. A reagent blank is necessary for the nickel procedure.
The Pour-Thru Cell can be used with 25-mL reagents only.
Total recoverable cobalt requires a prior digestion. See the Digestion section in the *Water Analysis Guide*.
For samples containing iron (Fe^{3+}), all of the powder must dissolve before mixing the sample in step 6.

Collect the following items:

Description	Quantity
EDTA reagent powder pillows	2
Phthalate-Phosphate reagent powder pillows	2
PAN indicator solution	1 mL
Water, deionized	25 mL
Cylinder, graduated mixing, 25-mL	2
Sample cells (*Instrument-specific information*)	2
Stopper for sample cells	2

See *Consumables and replacement items* for reorder information.

PAN method for powder pillows



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.

2. Prepared Sample:

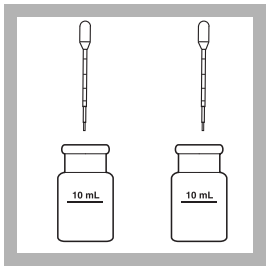
Fill a sample cell to the 10-mL mark with room-temperature sample.

3. Blank Preparation:

Fill a second sample cell to the 10-mL mark with room-temperature deionized water.

4. Add the contents of one Phthalate-Phosphate Reagent powder pillow to each cell. Swirl to completely dissolve.

PAN method for powder pillows (continued)



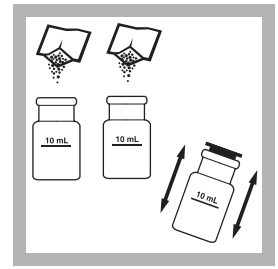
5. Use the plastic dropper provided to add 0.5 mL of 0.3% PAN Indicator Solution to each cell.

6. Use a stopper to close each cell.

Invert several times to mix.

7. Start the instrument timer. A three-minute reaction time will begin.

During the reaction period, the sample solution color may vary from green to dark red, depending on the chemical makeup of the sample. The deionized water blank should be yellow.

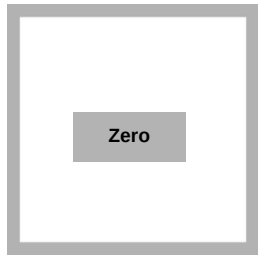


8. When the timer expires, add the contents of one EDTA Reagent powder pillow to each cell.

Use a stopper to close each cell.

Shake each cell to dissolve.

9. Wipe the blank and insert it into the cell holder.

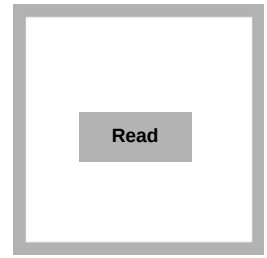


10. **ZERO** the instrument.

The display will show:

0.00 mg/L Co

11. Wipe the prepared sample and insert it into the cell holder.



12. **READ** the results in mg/L Co.

Interferences

Table 134 Interfering substances

Interfering substance	Interference level and treatments
Al^{3+}	32 mg/L
Ca^{2+}	1000 mg/L as $CaCO_3$
Cd^{2+}	20 mg/L
Cl^-	8000 mg/L
Cr^{3+}	20 mg/L
Cr^{6+}	40 mg/L
Cu^{2+}	15 mg/L

Table 134 Interfering substances (continued)

Interfering substance	Interference level and treatments
F^-	20 mg/L
Fe^{2+}	Interferes directly and must not be present
Fe^{3+}	10 mg/L
K^+	500 mg/L
Mg^{2+}	400 mg/L
Mn^{2+}	25 mg/L
Mo^{6+}	60 mg/L
Na^+	5000 mg/L
Pb^{2+}	20 mg/L
Zn^{2+}	30 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

- Collect samples in acid-washed plastic bottles.
- Adjust the sample pH to 2 or less with nitric acid* (about 5 mL per liter).
- Preserved samples can be stored up to six months at room temperature.
- Adjust the sample pH between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution* just before analysis. Do not exceed pH 8 as this may cause some loss of cobalt as a precipitate.
- Correct test results for volume additions.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Prepare a 1.00 mg/L cobalt standard solution as follows:

1. Dilute 10.0 mL of a 10-mg/L working stock solution to 100 mL in a volumetric flask. Prepare the 10-mg/L working stock solution daily by diluting 10.00 mL of Cobalt Standard Solution, 1000-mg/L as Co, to 1000 mL with deionized water.
2. To adjust the calibration curve using the reading obtained with the 1.00-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
110	1.00 mg/L Co	0.99–1.01 mg/L Co	0.01 mg/L Co

* See *Optional reagents and apparatus*.

Summary of method

After buffering the sample and masking any Fe^{3+} with pyrophosphate, the cobalt is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt, which can both be determined using the same sample.

Test results are measured at 620 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Cobalt Reagent Set, 10-mL (100 tests), includes:	—	—	2651600
(2) EDTA Reagent Powder Pillows	2	100/pkg	700599
(2) Phthalate-Phosphate Reagent Powder Pillows	2	100/pkg	2615199
(1) PAN Indicator Solution, 0.3%	1 mL	100 mL	2150232
Water, deionized	25 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Stopper, rubber	2	6/pkg	173106
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Cobalt Standard Solution, 1000-mg/L Co	100 mL	2150342

Optional reagents and apparatus

Description	Unit	Catalog number
Nitric Acid, 1:1	500 mL	254049
Sodium Hydroxide Standard Solution, 5.0 N	100 mL	245032
Pipet, volumetric, Class A, 10 mL	each	1451538
Flask, volumetric, Class A, 100 mL	each	1457442
Flask, volumetric, Class A, 1000 mL	each	1457453
Pipet Filter, Safety bulb	each	1465100
pH paper	100/pkg	2601300

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Edition 7

Color, True and Apparent

DOC316.53.01037

Platinum-Cobalt Standard Method[1, 2, 3]

Method 8025

15 to 500 units

Scope and Application: For water, wastewater and seawater; equivalent to NCASI method 253 and NCASI Method Color 71.01 for pulp and paper effluent using 465 nm (requires pH adjustment).

[1] Adapted from *Standard Methods for the Examination of Water* and *Wastewater and National Council for Air and Stream Improvement (NCASI) Methods Manual*.

[2] Adapted from *Wat. Res.* Vol. 30, No. 11, pp. 2771–2775, 1996.

[3] NCASI Method 253 approved at 40 CFR part 136.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 135 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

The NCASI procedure requires pH adjustment. Adjust the pH to 7.6 with 1.0 N HCl or 1.0 N NaOH. When adjusting the pH, if the overall volume change is greater than 1%, start over and use a stronger acid or base. Use program 125 when performing the NCASI procedure.
One powder pillow of pH 8 Buffer (sodium phosphate/potassium phosphate) may be added to 50 ml of sample prior to final pH adjustment to reduce sample dilution effects. Mix thoroughly to dissolve before making final pH adjustments.
To test for apparent color, omit steps 3–5 and step 7. Use unfiltered deionized water in step 6 and unfiltered sample in step 8.
For low level samples, use of a Pour-Thru Cell is recommended.

Collect the following items:

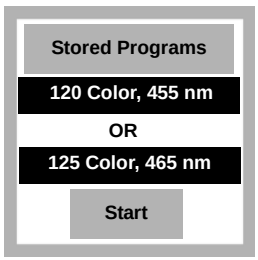
Description	Quantity
Buffer, pH 8.0 (Program 125)	1
Hydrochloric Acid Solution, 1.0 N (Program 125)	varies
Sodium Hydroxide, 1.00 N (Program 125)	varies
Water, deionized	100 mL
FIlter Apparatus: membrane filter, filter holder, filter flask and aspirator	1
Sample Cells (*Instrument-specific information*)	2

Collect the following items:

Description	Quantity
Stopper, rubber, one hole, No. 7	1
Tubing, rubber	1

See *Consumables and replacement items* for reorder information.

Plantinum-Cobalt method

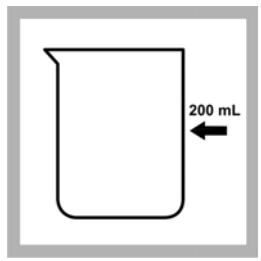


1. Select the test.

NCASI: Use Program 125 for the NCASI test.

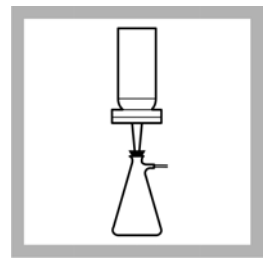
Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



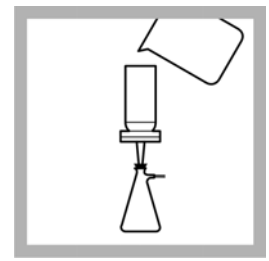
2. Collect 200 mL of sample in a 400-mL beaker.

NCASI: Adjust the pH as described in Test Preparation.

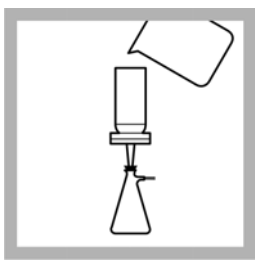


3. Assemble the filter apparatus (0.45 micron membrane filter, filter holder, filter flask and aspirator).

NCASI: The NCASI test prescribes a 0.8-micron filter. A 1.0 micron prefilter can be first used for difficult to filter samples.



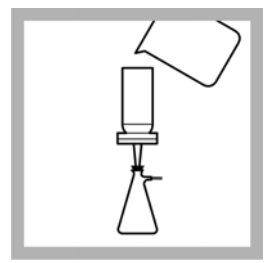
4. Filter about 50 mL of deionized water to rinse the filter. Discard the rinse water.



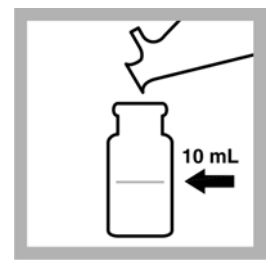
5. Filter another 50 mL of deionized water through the filter.

6. Blank Preparation: Fill a sample cell with 10-mL of filtered deionized water from step 5.

Discard the excess water in the flask.



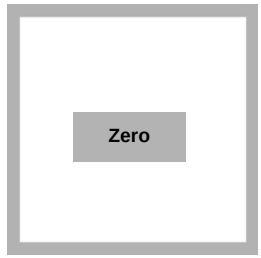
7. Filter about 50 mL of sample through the filter.



8. Prepared Sample: Fill a second sample cell with 10 mL of filtered sample.

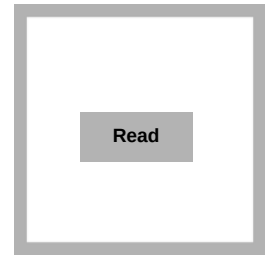
Plantinum-Cobalt method (continued)

9. Wipe the blank and insert it into the cell holder.



10. **ZERO** the instrument.
The display will show:
0 units Pt-Co

11. Wipe the prepared sample and insert it into the cell holder.



12. **READ** the results in mg/L Pt-Co.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, fill bottles completely and cap tightly.
- Avoid excessive agitation or prolonged contact with air.
- Samples can be stored for 24 hours by cooling to 4 °C (39 °F).
- Warm to room temperature before analysis.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Prepare a 250 platinum-cobalt units standard as follows:

1. Using Class A glassware, pipet 50.00 mL of a 500 Platinum-Cobalt Units Standard Solution into a 100-mL volumetric flask. Dilute to the 100 mL mark with deionized water.
2. To adjust the calibration curve using the reading obtained with the 250 unit Standard Solution, Select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
120	250 units Pt-Co	245–255 units Pt-Co	16 units Pt-Co
125	250 units Pt-Co	245–255 units Pt-Co	16 units Pt-Co

Summary of method

Color may be expressed as “apparent” or “true” color. The apparent color includes that from dissolved materials plus that from suspended matter. By filtering or centrifuging out the suspended materials, the true color can be determined. The procedure describes true color analysis. If apparent color is desired, it can be determined by measuring an unfiltered water sample. The same stored program is used for both forms of color.

The stored program is calibrated in color units based on the APHA-recommended standard of 1 color unit being equal to 1 mg/L platinum as chloroplatinate ion. Test results for Programs 120 and 125 are measured at 455 and 465 nm, respectively.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Buffer, pH 8.0	1	15/pK	1407995
Hydrochloric Acid Solution, 1.0 N	varies	1 L	2321353
Sodium Hydroxide, 1.00 N	varies	1 L	104553
Water, deionized	100 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Aspirator, Nalgene vacuum pump	1	each	213100
Filter, membrane, 47-mm, 0.8-microns, Program 125	1	100/pkg	2640800
Filter, membrane, 47-mm, 0.45-microns, Program 120	1	100/pkg	1353000
Flask, filtering, 500-mL	1	each	54649
Stopper, rubber, one hole, No. 7	1	6/pkg	211907
Tubing, rubber, 7.9 x 2.4 mm	varies	12 ft	56019
Filter Holder, 47 mm, 300 mL graduated	1	each	1352900
Beaker, 400 mL	1	each	50048
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards and apparatus

Description	Unit	Catalog number
Color Standard Solution, 500 platinum-cobalt units	1 L	141453
Color Standard Solution, 15 platinum-cobalt units	1 L	2602853
Color Standard Solution, 500 platinum-cobalt units, 10-mL Voluette® Ampules	16/pkg	141410
Filter, Glass Microfiber, 47-mm, 1.0 micron	100/pk	2551400
Flask, volumetric, Class A, 100 mL	each	1457442
Pipet, volumetric, Class A, 50.00 mL	each	1451541
Pipet Filler, safety bulb	each	1465100

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Edition 7

Color, ADMI

DOC316.53.01122

ADMI Weighted Ordinate Method[1]

Method 10048

(5 to 250 units Pt-Co)

Scope and Application: For water and wastewater where color characteristics are significantly different from platinum-cobalt standards, or for water and wastewater with color characteristics similar in hue to the standards. Turbid samples must be filtered prior to analysis.

[1] Adapted from Allen, et. al., 1973. Determination of color of water and wastewater by means of ADMI Color Values. *Proc. 28th Ind. Waste Conf.*, Purdue Univ., Eng. Ext. Ser. No. 142:661

Test preparation

The ADMI color method is available on the DR 5000 and DR 6000 spectrophotometer. One of three sample cell sizes can be used (refer to *Sample cell options*). Select the test that corresponds to the sample cells used (refer to step 1 of the procedure).

Table 136 Sample cell options

Program	Sample cell	Cell orientation
97	2495402	Fill line faces user
96	2095100	Clear side toward user
98	2629250	Clear side toward user

Before starting the test:

If the sample cannot be analyzed immediately, see *Sample collection, preservation and storage*.

Make pH adjustments with a minimum volume of acid or base. Make major adjustments with strong acid or base, then fine-tune with the 0.1 N solution.

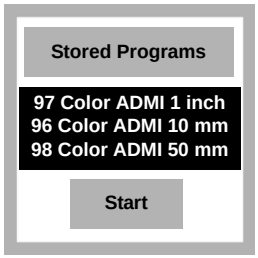
The sample cells shown in the procedure are a generic representation. Each of the three stored programs uses a cell of a different shape.

Collect the following items:

Description	Quantity
Sodium Hydroxide or Sulfuric Acid Solution, 10 N	varies
Sodium Hydroxide or Sulfuric Acid Solution, 0.100 N	varies
Water, deionized	50 mL
Beaker, 250 mL polypropylene	2
Cylinder, graduated, 100 mL polypropylene	1
Filter Apparatus: membrane filter, filter holder, filter flasks (2), tubing, stopper and aspirator	1
pH meter with electrode	1
Sample Cells, 1-inch square, 1 cm (10 mm) or 5 cm (50 mm)	2

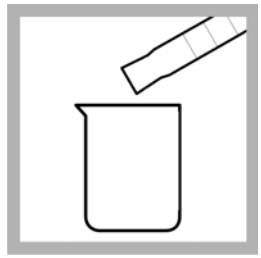
See *Consumables and replacement items* for reorder information.

ADMI weighted ordinate method



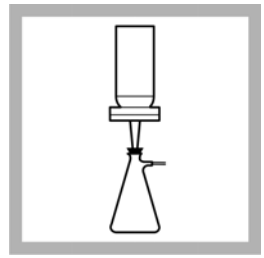
1. Select the test.

Insert the multi-cell adapter. Refer to *Sample cell options* for cell orientation.

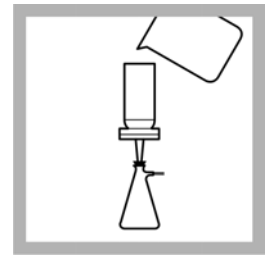


2. If the sample is not turbid, omit steps 3–6. Pour two 100-mL aliquots of sample into separate beakers. Adjust the pH of one of the aliquots to 7.6; leave the other aliquot as is.

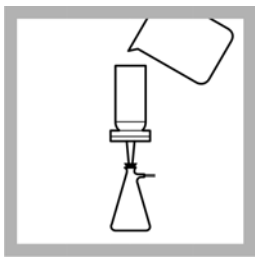
Use 10 N sodium hydroxide or concentrated sulfuric acid to adjust the pH. Use 0.1 N acid or base near the end point.



3. Assemble the filtering apparatus (0.45 micron membrane filter, filter holder, filter flask, and aspirator).



4. Rinse the filter by pouring about 50 mL of the original sample aliquot through the filter. Discard the rinse water.

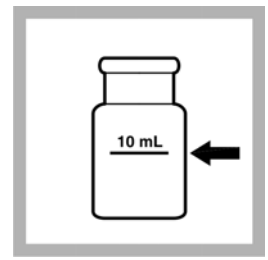


5. Pour another 50 mL of the original sample aliquot through the filter.

Label the flask “original”.

6. Repeat steps 3–5 for the pH adjusted sample and label it “pH adjusted”.

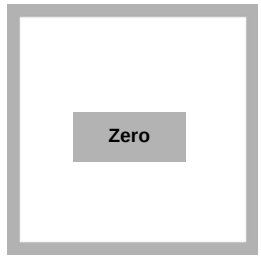
7. Prepared Sample: Fill a sample cell with the pH-adjusted sample. Discard the excess. Discard the excess water in the flask.



8. Blank Preparation: Fill a second sample cell with deionized water.

ADMI weighted ordinate method (continued)

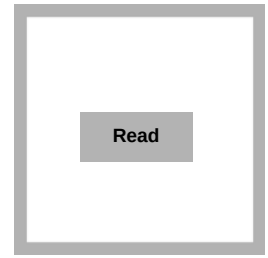
9. Wipe the blank and insert it into the cell holder. Close the light shield.



10. **ZERO** the instrument.

The instrument will read the percent transmittance (%T) at 10-nm intervals from 700 nm to 400 nm.

11. Wipe the prepared sample (pH adjusted) and insert it into the cell holder. Close the light shield.



12. **READ** the results. The instrument will read the percent transmittance (%T) at 10-nm intervals from 700 nm to 400 nm. When finished, the instrument will display the ADMI color value of the pH-adjusted sample.

Repeat steps 7–12 for the original sample.

For USEPA reporting, report both results.

Interferences

Turbidity interferes directly and must be removed using filtration.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.
- If prompt analysis is impossible, fill bottles completely and cap tightly.
- Avoid excessive agitation or prolonged contact with air.
- Samples can be stored for 24 hours by cooling to 4 °C (39 °F).
- Warm stored samples to room temperature before analysis.

Accuracy check

Standard solution method
Required for accuracy check:

- Color Standard Solution, 500 platinum-cobalt units
- Deionized water
- 100-mL Class A volumetric flask
- 20-mL Class A volumetric pipet and pipet bulb

1. Prepare a 100 platinum-cobalt units standard solution as follows:
   a. Pipet 20 mL of Color standard, 500 platinum-cobalt units, into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
2. Use this solution in place of the sample. Follow the *ADMI weighted ordinate method* test procedure. Omit filtration steps 3–6.
3. To adjust the calibration curve using the reading obtained with the standard solution, press **OPTIONS**>**MORE**>**STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Summary of method

Three properties describe color: hue, chroma and value. Hue is “color”, whether it be blue, red, green, yellow, etc. Chroma is color intensity (bright or dull). Value is the amount of color (light or dark). This method measures only the amount of color, or color value. It is independent of the hue and chroma.

This method determines the color value in a sample. Transmittance is measured from 400 to 700 nm and converted to a set of abstract numbers. These numbers describe the color as seen by an average human eye. They are converted to a single number that indicates the color value. This number is expressed on a scale used by the American Dye Manufacturers Institute (ADMI) to measure color value. The ADMI has adopted the Platinum-Cobalt standard of the American Public Health Association (APHA) as the standard for color value. Although this standard is yellow, the ADMI method works for all hues. Three calibrations are stored in the instrument. Each calibration corresponds to one of the three sample cell pathlengths.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Sodium Hydroxide Solution, 10 N	varies	500 mL	2545049
Sodium Hydroxide Standard Solution, 0.100 N	varies	1000 mL	19153
Sulfuric Acid, 10 N	varies	1000 mL	93153
Sulfuric Acid Standard Solution, 0.100 N	varies	100 mL MDB	20232H
Water, deionized	10 mL	4 liters	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 250-mL polypropylene	2	each	108046
Cylinder, graduated, 100-mL, polypropylene	1	each	108142
pH meter, with electrode	1	each	HQ11d
Sample cells, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Color Standard Solution, 500 platinum-cobalt units	1 L	141453

Optional reagents and apparatus

Description	Unit	Catalog number
Aspirator, Nalgene vacuum pump	each	213100
Filter Holder, 47-mm, 300-mL graduated	each	1352900
Filter, membrane, 47-mm, 0.45-microns	100/pkg	1353000
Flask, filtering, 500-mL	each	54649
Flask, volumetric, Class A, 100 mL	each	1457442
Pipet Filler, safety bulb	each	1465100
Pipet, volumetric, Class A, 20.00 mL	each	1451520
Stopper, rubber, one hole, No. 7	6/pkg	211907
Tubing, rubber	12 ft	56019

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Edition 7

Copper

DOC316.53.01039

USEPA[1] Bicinchoninate Method[2]

Method 8506
Method 8026

(0.04 to 5.00 mg/L) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater[3]; Method 8506 USEPA approved for reporting wastewater analysis (digestion required).[4]

[1] Approved, USEPA and Standard Method 3500 Cu C or E

[2] Adapted from Nakano, S., *Yakugaku Zasshi*, 82 486-491 (1962) [*Chemical Abstracts,* 58 3390e (1963)].

[3] Pretreatment required; see *Interferences (Using Powder Pillows).*

[4] *Federal Register*, 45 (105) 36166 (May 29, 1980).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 137 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

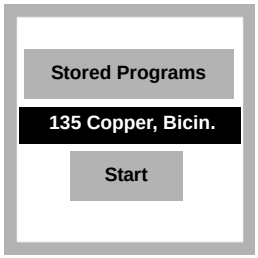
Digestion is required for determining total copper.
Adjust the pH of acid-preserved samples to 4–6 with 8 N KOH before analysis.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
If copper is present, the sample will turn purple when it mixes with the reagent powder.
Accuracy is not affected by undissolved reagent.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
CuVer® 1 Copper Reagent powder pillow	1
Sample Cells, powder pillow test (*Instrument-specific information*)	2
AccuVac Test:	
CuVer® 2 Copper Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample Cell, AccuVac test (*Instrument-specific information*)	1
Stopper for 18 mm tube	1

See *Consumables and replacement items* for reorder information.

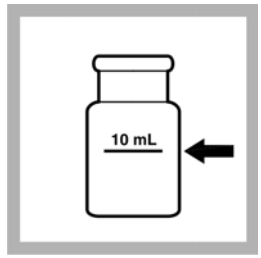
Bicinchoninate method for powder pillows (Method 8506)



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



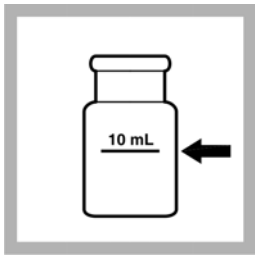
2. **Prepared Sample:** Fill a cell with 10 mL of sample.

3. Add the contents of one CuVer® 1 Copper Reagent powder pillow to the prepared sample cell. Swirl sample cell to mix.

Use a CuVer 2 Copper Reagent powder pillow for samples containing high levels of aluminum, iron and hardness. A 25-mL sample cell is required (*Interfering substances and suggested treatments for powder pillows*).

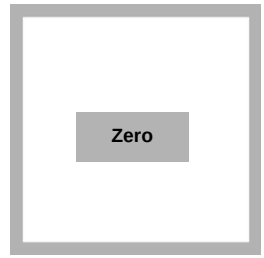
4. Start the instrument timer. A two-minute reaction period will begin.

Bicinchoninate method for powder pillows (Method 8506) (continued)



5. Blank Preparation: When the timer expires, fill a second sample cell with 10 mL of sample.

6. Insert the blank into the cell holder.



7. **ZERO** the instrument.

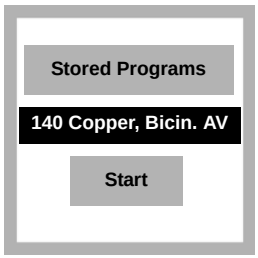
The display will show:

0.00 mg/L Cu

8. Within 30 minutes after the timer expires, insert the prepared sample into the cell holder.

READ the results in mg/L Cu.

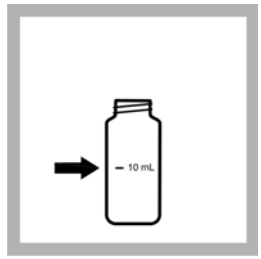
Bicinchoninate method for AccuVac® Ampuls (Method 8026)



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a round sample cell with 10 mL of sample.

3. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill a CuVer 2 AccuVac Ampul with sample from the beaker. Keep the tip immersed while the Ampul fills completely.

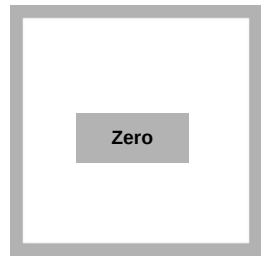
4. Place the stopper and quickly invert the Ampul several times to mix. Wipe off all liquid and fingerprints with a cloth or soft paper towel.

Bicinchoninate method for AccuVac® Ampuls (Method 8026) (continued)

5. Start the instrument timer.

A two-minute reaction period will begin.

6. When the timer expires, wipe the blank and insert it into the cell holder.



7. **ZERO** the instrument.

The display will show:

0.00 mg/L Cu

8. Within 30 minutes after the timer expires, wipe the Ampul and insert it into the cell holder.

READ the results in mg/L Cu.

Interferences

The *Interfering substances and suggested treatments for powder pillows* table suggests treatments for powder pillows. The *Interfering substances and suggested treatments for AccuVac Ampuls* tables suggests treatments for AccuVac Ampuls. To differentiate free copper from that complexed to EDTA or other complexing agents, use a 25-mL sample cell and Free Copper Reagent Powder Pillow instead of the CuVer 1 Powder Pillow in step 3. Add a Hydrosulfite Reagent Powder Pillow to the same sample and re-read the result. This result will include the total dissolved copper (free and complexed). Unlike CuVer 1 Reagent, CuVer 2 Reagent Powder Pillows and AccuVac Ampuls react directly with copper, which is complexed by chelants such as EDTA.

Table 138 Interfering substances and suggested treatments for powder pillows

Interfering substance	Interference levels and treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution drop-wise until sample pH is above 4. Continue with step 3.
Aluminum, Al^{3+}	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in step 3. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample volume.
Cyanide, CN^{-}	Prevents full color development. Before adding the CuVer 1 Powder Pillow Reagent, add 0.2 mL of formaldehyde to the 10-mL sample. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in step 3. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample volume.
Iron, Fe^{3+}	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in step 3. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample volume.
Silver, Ag^{+}	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

Table 139 Interfering substances and suggested treatments for AccuVac Ampuls

Interfering substance	Interference levels and treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution drop-wise until sample pH is above 4. Continue with step 3.
Aluminum, Al^{3+}	Reagents accommodate high levels.
Cyanide, CN^-	Prevents full color development. Add 0.5 mL of formaldehyde per 25-mL of sample before using CuVer 2 Reagent AccuVac Ampul. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Reagents accommodate high levels.
Iron, Fe^{3+}	Reagents accommodate high levels.
Silver, Ag^+	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

Sample collection, preservation and storage

- Collect samples in acid-cleaned glass or plastic containers.
- Adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter).
- Store preserved samples up to six months at room temperature.
- Before analysis, adjust the pH to 4–6 with 8 N Potassium Hydroxide. Do not exceed pH 6, as copper may precipitate.
- Correct the test result for volume additions.
- If only dissolved copper is to be determined, filter the sample before acid addition.

Accuracy check

Required for accuracy check:

- Copper Standard Solution, 100 mg/L Cu
- TenSette Pipet and Pipet Tips
- Mixing cylinders (3)
- Beakers (3)

Standard additions method (sample spike)

1. Prepare a 12.5 mg/L Copper standard by pipetting 5.0 mL of Copper standard solution, 100mg/L into a 50 mL mixing cylinder. Dilute the solution to 40 mL, stopper and mix.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select Options>More>Standard Additions from the instrument menu.
4. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Press the timer icon. After the timer expires, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Press the timer icon. After the timer expires, read the result.

7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Press the timer icon. After the timer expires, read the result. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac Ampuls, fill three mixing cylinders with 50-mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of Copper Voluette Ampule Standard, 75-mg/L Cu. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by pressing* ***READ****. Each addition should reflect approximately 100% recovery.*

8. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view relationships between the sample spikes and the “Ideal Line” of 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Prepare a 4.00-mg/L Standard as follows:

1. Using Class A glassware, pipet 4.00 mL of Copper Standard Solution, 100-mg/L as Cu, into a 100-mL volumetric flask. Dilute to volume with deionized water, stopper and invert to mix. Perform the procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 4.00-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
135	1.00 mg/L Cu	0.97–1.03 mg/L Cu	0.04 mg/L Cu
140	1.00 mg/L Cu	0.97–1.03 mg/L Cu	0.03 mg/L Cu

Summary of method

Copper in the sample reacts with a salt of bicinchoninic acid contained in CuVer 1 or CuVer 2 Copper Reagent to form a purple colored complex in proportion to the copper concentration. Test results are measured at 560 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
CuVer® 1 Copper Reagent Powder Pillows	1	100/pkg	2105869
OR			
CuVer® 2 Copper Reagent AccuVac® Ampuls	1	25/pkg	2504025

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Stopper for 18 mm tube	1	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Copper Standard Solution, 100-mg/L as Cu	100 mL	12842
Copper Voluette® Ampule Standard, 75-mg/L as Cu, 10-mL	16/pkg	1424710
Metals Drinking Water Standard, LR for Cu, Fe, Mn	500 mL	2833749
Metals Drinking Water Standard, HR for Cu, Fe, Mn	500 mL	2833649

Optional reagents and apparatus

Description	Unit	Catalog number
Beaker, 50-mL	each	500-41H
CuVer 2 Copper Reagent Powder Pillow	100/pkg	2188299
Cylinder, mixing,. 50 mL	each	189641
Formaldehyde, ACS	100 mL MDB	205932
Nitric Acid, concentrated	500 mL	15249
Potassium Chloride Solution	100 mL	76542
Potassium Hydroxide Standard Solution, 8 N	100 mL MDB	28232H
Reagent Set for Free and Total Copper, includes:	each	2439200
Hydrosulfite Reagent Powder Pillows	100/pkg	2118869
Free Copper Reagent Powder Pillows	100/pkg	2182369
Sample Cells, 25 mLmatched, 1” square	2/pkg	2612602
AccuVac Snapper	each	2405200
Ampule Breaker	each	2196800
Sample Cells, 25 mm round	6/pkg	2401906

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Edition 7

Copper

DOC316.53.01038

Porphyrin Method[1] **Method 8143**

LR (1 to 210 µg/L) **Powder Pillows**

Scope and Application: For water, wastewater and sea water

[1] Adapted from Ishii and Koh, *Bunseki Kagaku*, 28 (473), 1979

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 140 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

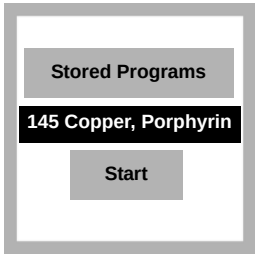
Digestion is required for determining total copper.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Wash all glassware with detergent. Rinse with tap water. Rinse again with 1:1 Nitric Acid Solution. Rinse a third time with copper-free, deionized water.
If samples contain high levels of metals, a slight metallic deposit or yellow buildup may form in the sample cell. Wash the cell as described above.

Collect the following items:

Description	Quantity
Copper Masking Reagent Powder Pillows	1
Porphyrin 1 Reagent Powder Pillows	2
Porphyrin 2 Reagent Powder Pillows	2
Nitric Acid Solution, 1:1	varies
Sample Cells (*Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

Porphyrin method



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.

2. FIll two sample cells with 10 mL of sample.

3. Blank Preparation: Add the contents of one Copper Masking Reagent powder pillow to one of the sample cells to create the blank. Swirl to dissolve.

4. Add the contents of one Porphyrin 1 Reagent powder pillow to each sample cell.

Swirl to dissolve.

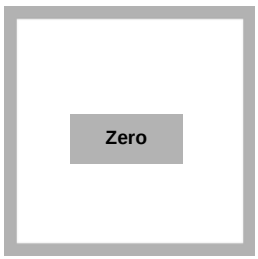
5. Add the contents of one Porphyrin 2 Reagent powder pillow to each sample cell.

6. Swirl to dissolve.

If copper is present the sample will briefly turn blue, then return to yellow.

7. Start the instrument timer. A three-minute reaction period will begin.

8. When the timer expires insert the blank into the cell holder.

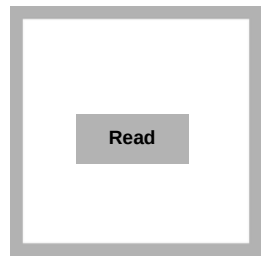


9. **ZERO** the instrument.

The display will show:

0 µg/L Cu

10. Insert the prepared sample into the cell holder.



11. **READ** the results in µg/L Cu.

Interferences

Table 141 Interfering substances and levels

Interfering substance	Interference levels and treatments
Aluminum, Al^{3+}	60 mg/L
Cadmium, Cd^{2+}	10 mg/L
Calcium, Ca^{2+}	1500 mg/L
Chelating agents	Interfere at all levels unless either the Digesdahl or vigorous digestion is performed
Chloride, Cl^{-}	90,000 mg/L
Chromium, Cr^{6+}	110 mg/L
Cobalt, Co^{2+}	100 mg/L
Fluoride, F^{-}	30,000 mg/L
Iron, Fe^{2+}	6 mg/L
Lead, Pb^{2+}	3 mg/L
Magnesium	10,000 mg/L
Manganese	140 mg/L
Mercury, Hg^{2+}	3 mg/L
Molybdenum	11 mg/L
Nickel, Ni^{2+}	60 mg/L
Potassium, K^{+}	60,000 mg/L
Sodium, Na^{+}	90,000 mg/L
Zinc, Zn^{2+}	9 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

- Collect samples in acid-washed plastic bottles.
- To preserve, adjust the pH to 2 or less with nitric acid (about 5 mL per liter).
- Store preserved samples up to six months at room temperature.
- Before testing, adjust the pH of the preserved sample to between 2 and 6. If the sample is too acidic, adjust the pH with 5.0 N Sodium Hydroxide Standard Solution*.
- Correct test results for volume additions.

Accuracy check

Required for accuracy check:

- Copper Standard Solution, 4 mg/L Cu Pour-Rite Ampules
- TenSette Pipet and Pipet Tips

* See *Optional reagents and apparatus*.

Standard additions method (sample spike)

4. After reading test results, leave the sample cell (unspiked sample) in the instrument.
5. Select Options>More>Standard additions from the instrument menu.
6. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
7. Fill eight sample cells with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL from a 4-mg/L Pour-Rite Ampule, to two of the sample cells. Then pipet 0.2 mL of the standard solution into two more cells. Finally, pipet 0.3 mL of the standard solution into two more cells.
8. Analyze each standard addition sample as described in the procedure above, using one of the two spiked samples in each set as the blank. Accept each standard additions reading by pressing **READ**. The copper concentration reading should reflect approximately 100% recovery.
9. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. To assure the accuracy of the test, prepare a 150-µg/L copper standard by pipetting 15.00 mL of Copper Standard Solution, 10.0-mg/L Cu, into a 1000-mL volumetric flask.
2. Dilute to the mark with copper-free, reagent-grade water. Prepare this solution daily. Perform the copper procedure as described above.
3. To adjust the calibration curve using the reading obtained with the 150-µg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
145	50 µg/L Cu	47–53 µg/L Cu	1 µg/L Cu

Summary of method

The porphyrin method is very sensitive to trace amounts of free copper. The method is free from most interferences and does not require any sample extraction or concentration before analysis. Interferences from other metals are eliminated by the copper masking reagent. The porphyrin indicator forms an intense, yellow-colored complex with any free copper present in sample. Test results are measured at 425 nm.

Consumables and replacement items

Required reagents and apparatus

Description	Quantity/Test	Unit	Catalog number
Copper Reagent Set (100 tests), includes:	—	—	2603300
(1) Copper Masking Reagent Powder Pillows	1	100/pkg	2603449
(2) Porphyin 1 Reagent Powder Pillows	2	100/pkg	2603549
(2) Porphyrin 2 Reagent Powder Pillows	2	100/pkg	2603649
Nitric Acid Solution, 1:1	varies	500 mL	254049
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Copper Standard Solution, 4 mg/L, 2 mL Pour-Rite Ampules	20/pkg	2605720
Copper Standard Solution, 10-mg/L Cu	100 mL	12932
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Sodium Hydroxide Standard Solution, 5.0 N MDB	100 mL	245032
Tensette Pipet, 0.1–1.0	each	1970001
Tips for Tensette Pipet	50/pkg	2185696
Pipet, Volumetric, Class A, 15 mL	each	1451539
Flask, Volumetric, Class A, 1000 mL	each	1457453
Pipet Filler, Safety Bulb	each	1465100
Sample Cells, 1" square matched set	8/pkg	2495408
pH paper	100/pkg	2601300

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Edition 7

Cyanide

DOC316.53.01040

Pyridine-Pyrazalone Method[1] **Method 8027**

(0.002 to 0.240 mg/L CN^-) **Powder Pillows**

Scope and Application: For water, wastewater and seawater.

[1] Adapted from Epstein, Joseph, *Anal. Chem.* 19(4), 272 (1947).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 142 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test

The sample cell shown is a generic representation. Refer to *Instrument-specific information* for the correct sample cell and adapter configuration.
Use a water bath to maintain the optimum temperature for the reaction in this test (25 °C). Samples at less than 23 °C require longer reaction times and samples at greater than 25 °C yield low results.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
The timing for steps 3–10 is critical. You may find it useful to open the necessary reagents before starting this sequence.
All samples to be analyzed for cyanide should be treated by acid distillation except when experience has shown that there is no difference in results obtained with or without distillation. See *Acid distillation*.
See *Pollution prevention and waste management* for proper disposal of solutions containing cyanide.

Collect the following items:

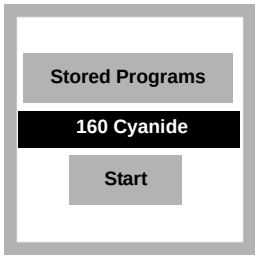
Description	Quantity
CyaniVer® Cyanide 3 Reagent Powder Pillow	1
CyaniVer® Cyanide 4 Reagent Powder Pillow	1
CyaniVer® Cyanide 5 Reagent Powder Pillow	1
Cylinder, graduated, 10-mL	1
Sample Cells, 1-inch square glass	2

Collect the following items:

Description	Quantity

See *Consumables and replacement items* for reorder information.

Pyridine-Pyrazalone

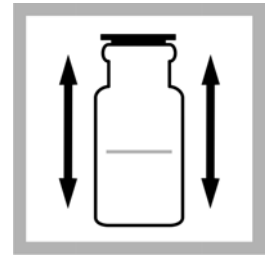


1. Select the test.

Insert the adapter, if required (*Instrument-specific information*).

2. Using a graduated cylinder, fill a sample cell with 10 mL of sample.

3. Prepared Sample: Add the contents of one CyaniVer 3 Cyanide Reagent Powder Pillow. Cap the cell.



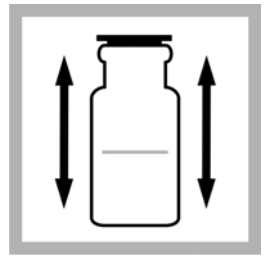
4. Shake the sample cell for 30 seconds.

5. Leave the sample cell undisturbed for an additional 30 seconds.

6. Add the contents of one CyaniVer 4 Cyanide Reagent Powder Pillow.

Cap the sample cell.

Undissolved reagent will not affect accuracy.



7. Shake the sample for 10 seconds. Immediately proceed to step 8. (Delaying the addition of the CyaniVer 5 for more than 30 seconds will produce low test results.)

8. Add the contents of one CyaniVer 5 Cyanide Reagent Powder Pillow.

Cap the sample cell.

9. Shake the cell vigorously.

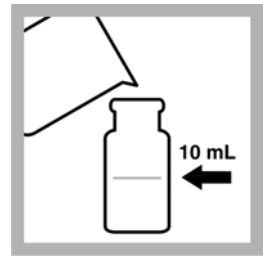
If cyanide is present, a pink color will develop.

10. Set the timer.

A 30-minute reaction period will begin.

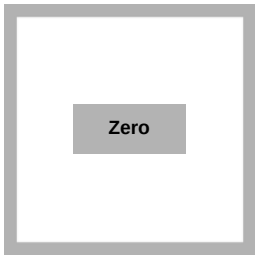
The solution will turn from pink to blue.

Samples less than 25 °C require a longer reaction time. Samples greater than 25 °C give low test results.



11. Blank Preparation: When the timer expires, fill a second sample cell with 10 mL of sample.

12. Wipe the blank and insert it into the cell holder.



13. ZERO the instrument.

The display will show:

0.000 mg/L CN^-.

14. Wipe the prepared sample and insert it into the cell holder. Review the user manual for cell orientation.

Results are in mg/L CN^-.

Pollution prevention and waste management

Special considerations for materials containing cyanide
Samples analyzed by this procedure may contain cyanide, which is regulated as reactive (D003) waste by the federal RCRA. It is imperative these materials be handled safely to prevent the release of hydrogen cyanide gas (an extremely toxic material with the smell of almonds). Most cyanide compounds are stable and can be safely stored for disposal in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes which may contain lower pH materials such as acids or even water.

In the event of a spill or release, special precautions must be taken to prevent exposure to hydrogen cyanide gas. The following steps may be taken to destroy the cyanide compounds in the event of an emergency:

- Use a fume hood or supplied air or self contained breathing apparatus.
- While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).
- Maintain a strong excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.
- Neutralize and flush the solution down the drain with a large excess of water.

Note: *If the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Never flush hazardous wastes down the drain.*

Interferences

Table 143 Interfering substances

Interfering substance	Interference levels and treatments
Chlorine	Large amounts of chlorine in the sample will cause a milky white precipitate after the addition of the CyaniVer® 5 Reagent. If chlorine or other oxidizing agents are known to be present, pretreat the sample before testing using the procedure in this table for oxidizing agents.
Metals	Nickel or cobalt in concentrations up to 1 mg/L do not interfere. Eliminate the interference from up to 20 mg/L copper and 5 mg/L iron by adding the contents of one HexaVer Chelating Reagent Powder Pillow to the sample and then mixing before adding the CyaniVer 3 Cyanide Reagent Powder Pillow in step 3. Prepare a reagent blank of deionized water and reagents to zero the instrument in step 12.
Oxidizing agents	**1.** Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution. Count the number of drops of acid added. **2.** Add two drops of Potassium Iodide Solution and two drops of Starch Indicator Solution to the sample. Swirl to mix. The sample will turn blue if oxidizing agents are present. **3.** Add Sodium Arsenite Solution drop-wise until the sample turns colorless. Swirl the sample thoroughly after each drop. Count the number of drops. **4.** Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in step 1. **5.** Subtract one drop from the amount of Sodium Arsenite Solution added in step 3. Add this amount to the sample and mix thoroughly. Continue with step 2 of the cyanide procedure.
Reducing agents	**1.** Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution. Count the number of drops added. **2.** Add four drops of Potassium Iodide Solution and four drops of Starch Indicator Solution to the sample. Swirl to mix. The sample should be colorless. **3.** Add Bromine Water drop-wise until a blue color appears. Swirl the sample thoroughly after each addition. Count the number of drops. **4.** Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in step 1. **5.** Add the total number of drops of Bromine Water counted in step 3 to the sample and mix thoroughly. **6.** Continue with step 2 of the cyanide procedure.

Table 143 Interfering substances (continued)

Interfering substance	Interference levels and treatments
Turbidity	Large amounts of turbidity will cause high readings. Use filter paper and a funnel to filter highly turbid water samples before use in steps 1 and 11. The test results should then be recorded as soluble cyanide.

Sample collection, preservation and storage

- Collect samples in glass or plastic bottles and analyze as quickly as possible.
- The presence of oxidizing agents, sulfides and fatty acids can cause the loss of cyanide during sample storage. Samples containing these substances must be pretreated as described below before preservation with sodium hydroxide. If the sample contains sulfide and is not pretreated, it must be analyzed within 24 hours.
- Preserve the sample by adding 4.0 mL of 5.0 N Sodium Hydroxide Standard Solution to each liter (or quart) of sample, using a glass serological pipet and pipet filler.
- Check the sample pH; 4-mL of sodium hydroxide is usually enough to raise the pH of most water and wastewater samples to 12. Add more 5.0 N Sodium Hydroxide if necessary.
- Store the samples at 4 °C (39 °F) or less. Samples preserved in this manner can be stored for 14 days.
- Before testing, samples preserved with 5.0 N Sodium Hydroxide or samples that are highly alkaline due to chlorination treatment processes or sample distillation procedures should be adjusted to approximately pH 7 with 2.5 N Hydrochloric Acid Standard Solution.
- Correct the volume when significant amounts of preservative are used.

Oxidizing agents
Oxidizing agents such as chlorine decompose cyanides during storage. To test for their presence and to eliminate their effect, pretreat the sample as follows:

1. Take a 25-mL portion of the sample and add one drop of 10-g/L m-Nitrophenol Indicator Solution. Swirl to mix.
2. Add 2.5 N Hydrochloric Acid Standard Solution drop-wise until the color changes from yellow to colorless. Swirl the sample thoroughly after the addition of each drop.
3. Add two drops of Potassium Iodide Solution, 30-g/L and two drops of Starch Indicator Solution, to the sample. Swirl to mix. The solution will turn blue if oxidizing agents are present.
4. If step 3 suggests the presence of oxidizing agents, add two level, 1-g measuring spoonfuls of Ascorbic Acid per liter of sample.
5. Withdraw a 25-mL portion of sample treated with ascorbic acid and repeat steps 1 to 3. If the sample turns blue, repeat steps 4 and 5.
6. If the 25-mL sample remains colorless, preserve the remaining sample to pH 12 for storage with 5 N Sodium Hydroxide Standard Solution.
7. Perform the procedure given under Interfering Substances and Levels, Reducing Agents, to eliminate the effect of excess ascorbic acid, before following the cyanide procedure.

Sulfides

Sulfides will quickly convert cyanide to thiocyanate (SCN^-). To test for the presence of sulfide and eliminate its effect, pretreat the sample as follows:

1. Place a drop of sample on a disc of Hydrogen Sulfide Test Paper that has been wetted with pH 4 Buffer Solution.
2. If the test paper darkens, add a 1-g measuring spoon of Lead Acetate to the sample. Repeat step 1.
3. If the test paper continues to turn dark, keep adding Lead Acetate until the sample tests negative for sulfide.
4. Filter the lead sulfide precipitate through Filter Paper and a Funnel. Preserve the sample for storage with 5 N Sodium Hydroxide Standard Solution or neutralize to a pH of 7 for analysis.

Fatty acids

CAUTION
Perform this operation under a ventilation hood and complete as quickly as possible.

When distilled, fatty acids will pass over with cyanide and under the alkaline conditions of the absorber, will form soaps. If the presence of fatty acid is suspected, use the following pretreatment before preserving samples with sodium hydroxide.

1. Acidify 500 mL of sample to pH 6 or 7 with a 4:1 dilution of glacial Acetic Acid.
2. Pour the sample into a 1000-mL separation funnel and add 50 mL of Hexane.
3. Stopper the funnel and shake for one minute. Allow the layers to separate.
4. Drain off the lower, sample layer into a 600-mL beaker. If the sample is to be stored, add enough 5 N Sodium Hydroxide Standard Solution to raise the pH above 12.

Acid distillation

All samples to be analyzed for cyanide should be treated by acid distillation except when experience has shown that there is no difference in results obtained with or without distillation. With most compounds, a one-hour reflux is adequate.

If thiocyanate is present in the original sample, a distillation step is absolutely necessary as thiocyanate causes a positive interference. High concentrations of thiocyanate can yield a substantial quantity of sulfide in the distillate. The “rotten egg” smell of hydrogen sulfide will accompany the distillate when sulfide is present. The sulfide must be removed from the distillate prior to testing.

If cyanide is not present, the amount of thiocyanate can be determined. The sample is not distilled and the final reading is multiplied by 2.2. The result is mg/L SCN^-.

The distillate can be tested and treated for sulfide after the last step of the distillation procedure by using the following lead acetate treatment procedure.

1. Place a drop of the distillate (already diluted to 250 mL) on a disc of Hydrogen Sulfide Test Paper that has been wetted with pH 4 Buffer Solution.
2. If the test paper darkens, add 2.5 N Hydrochloric Acid Standard Solution by drops to the distillate until a neutral pH is obtained.
3. Add a 1-g measuring spoon of Lead Acetate to the distillate and mix. Repeat step 1.
4. If the test paper continues to turn dark, keep adding lead acetate until the distillate tests negative for sulfide. Filter the black lead sulfide precipitate through filter paper and a funnel. Neutralize the liquid filtrate to pH 7 and immediately analyze for cyanide.

Distillation procedure

The following steps describe the distillation process using distillation apparatus and cyanide glassware offered by the manufacturer:

1. Set up the distillation apparatus for cyanide recovery, leaving off the thistle tube. Refer to the Distillation Apparatus Manual. Turn on the water and make certain it is flowing steadily through the condenser.
2. Fill the distillation apparatus cylinder to the 50-mL mark with 0.25 N Sodium Hydroxide Standard Solution.
3. Fill a clean 250-mL graduated cylinder to the 250-mL mark with sample and pour it into the distillation flask. Place a stirring bar into the flask and attach the thistle tube.
4. Arrange the vacuum system as shown in the Distillation Apparatus Manual, but do not connect the vacuum tubing to the gas bubbler. Turn on the water to the aspirator to full flow and adjust the flow meter to 0.5 SCFH.
5. Connect the vacuum tubing to the gas bubbler, making certain that air flow is maintained (check the flow meter) and that air is bubbling from the thistle tube and the gas bubbler.
6. Turn the power switch on and set the stir control to 5. Using a 50-mL graduated cylinder, pour 50 mL of 19.2 N Sulfuric Acid Standard Solution* through the thistle tube and into the distillation flask.
7. Using a water bottle, rinse the thistle tube with a small amount of deionized water.
8. Allow the solution to mix for three minutes; then add 20 mL Magnesium Chloride Reagent* through the thistle tube and rinse again. Allow the solution to mix for 3 more minutes.
9. Verify that there is a constant flow of water through the condenser.
10. Turn the heat control to 10.
11. Carefully monitor the distillation flask at this point in the procedure. Once the sample begins to boil, slowly lower the air flow to 0.3 SCFH. If the contents of the distillation flask begin to back up through the thistle tube, increase the air flow by adjusting the flow meter until the contents do not back up through the thistle tube. Boil the sample for one hour.
12. After one hour, turn off the still, but maintain the air flow for 15 minutes more.
13. After 15 minutes, remove the rubber stopper on the 500-mL vacuum flask to break the vacuum and turn off the water to the aspirator. Turn off the water to the condenser.
14. Remove the gas bubbler/cylinder assembly from the distillation apparatus. Separate the gas bubbler from the cylinder and pour the contents of the cylinder into a 250-mL, Class A volumetric flask. Rinse the gas bubbler, cylinder and J-tube connector with deionized water and add the washings to the volumetric flask.
15. Fill the flask to the mark with deionized water and mix thoroughly. Neutralize the contents of the flask and analyze for cyanide.

Accuracy check

Standard solutions method

CAUTION
Cyanides and their solutions and the hydrogen cyanide liberated by acids, are very poisonous. Both the solutions and the gas can be absorbed through the skin.

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Potassium Cyanide
- Deionized water

Prepare a 100-mg/L cyanide stock solution weekly as follows:

1. Dissolve 0.2503 grams of Potassium Cyanide in deionized water and dilute to 1000 mL.
2. Immediately before use, prepare a 0.200 mg/L cyanide working solution by diluting 2.00 mL of the 100 mg/L stock solution to 1000 mL using deionized water.
3. To adjust the calibration curve using the reading obtained with the 0.200 mg/L standard solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Point of Curve	Concentration
160	0.100 mg/L CN^-	0.090–0.110 mg/L CN^-	Entire Range	0.002 mg/L CN^-

Summary of method

The Pyridine-Pyrazalone method used for measuring cyanide gives an intense blue color with free cyanide. A sample distillation is required to determine cyanide from transition and heavy metal cyanide complexes. Test results are measured at 612 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Cyanide Reagent Set, includes:	—	—	2430200
(1) CyaniVer® 3 Cyanide Reagent Powder Pillow	1	100/pkg	2106869
(1) CyaniVer® 4 Cyanide Reagent Powder Pillow	1	100/pkg	2106969
(1) CyaniVer® 5 Cyanide Reagent Powder Pillow	1	100/pkg	2107069

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 10-mL	1	each	50838
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stopper, poly, hollow	—	6/pkg	1448000

Recommended standards

Description	Unit	Cat. No.
Potassium Cyanide, ACS	125 g	76714
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Acetic Acid, ACS	500 mL	10049
Ascorbic Acid	100 g	613826
Bromine Water, 30 g/L	29 mL	221120
Buffer Solution, pH 4	500 mL	1222349
Filter Paper, 12.5 cm	100/pkg	189457
Funnel, 65 mm	each	108367
Hexane Solution, ACS	500 mL	1447849
HexaVer Chelating Reagent Powder Pillow	100/pkg	24399
Hydrochloric Acid Standard Solution, 2.5 N	100 mL MDB	141832
Hydrogen Sulfide Test Paper	100/pkg	2537733
m-Nitrophenol Indicator Solution, 10 g/L	100 mL MDB	247632
Magnesium Chloride Reagent	1 L	1476253
Potassium Iodide Solution, 30 g/L	100 mL MDB	34332
Sodium Arsenite Solution, 5 g/L	100 mL	104732
Sodium Hydroxide Standard Solution, 0.25 N	1000 mL	1476353
Sodium Hydroxide Standard Solution, 5.0 N	1 L	245053
Starch Indicator Solution	100 mL MDB	34932
Sulfuric Acid Standard Solution, 19.2 N	500 mL	203849
Cyanide Glassware	each	2265800
Distillation Apparatus, 115 VAC	each	2274400

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Distillation Apparatus, 230 VAC	each	2274402
Distillation Apparatus Set	each	2265300
Pipet, seriological, 5 mL	each	53237
Pipet filler, Safety bulb	each	1465100
pH paper	100/pkg	2601300
Spoon, measuring, 1 g	each	51000
Thermometer, -10–225 °C	each	2635700

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Edition 7

Cyanuric Acid

DOC316.53.01183

Turbidimetric Method **Method 8139**

5 to 50 mg/L **Powder Pillows**

Scope and Application: For water.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 144 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

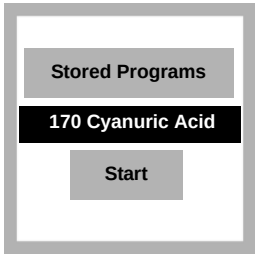
Filter highly turbid samples with filter paper and a funnel.
Clean sample cells with soap, water, and a brush soon after each test to avoid a build-up of film on the sample cell.
Do not use the Pour-Thru Cell with this procedure.

Collect the following items:

Description	Quantity
Bottle, mixing, square glass	1
Cyanuric Acid 2 Reagent Powder Pillow	1
Sample cells (See *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

Turbidimetric method



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

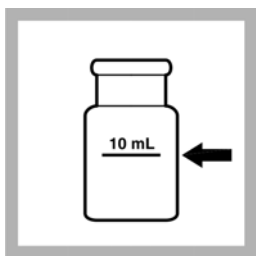
2. Fill a mixing bottle with 25 mL of sample.

3. Prepared Sample: Add the contents of one Cyanuric Acid 2 Reagent powder pillow. Swirl to mix.

After adding the reagent a white turbidity will form if cyanuric acid is present.

4. Start the instrument timer.

A three-minute reaction period will begin.



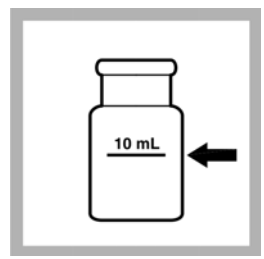
5. Blank Preparation: Fill a sample cell with 10 mL of sample from the mixing bottle.

6. Wipe and insert the blank sample cell into the cell holder.

ZERO the instrument.

The display will show:

0 mg/L Cyan Acid



7. When the timer expires, fill a second sample cell with 10 mL of prepared sample from the mixing bottle.

8. Within seven minutes after the timer expires, insert the prepared sample into the cell holder.

READ the results in mg/L Cyanuric Acid.

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Samples must be analyzed within 24 hours.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Dissolve 1.000 gram of cyanuric acid in 1 liter of deionized water to make a 1000-mg/L solution. Cyanuric acid is difficult to dissolve; it may take several hours to completely dissolve. This solution is stable for several weeks.
2. Dilute 3.00 mL of the 1000-mg/L solution to 100 mL with deionized water to make a 30-mg/L solution. Prepare fresh daily. Testing the 30-mg/L solution should give test results of about 30 mg/L cyanuric acid.

3. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity	
			Portion of curve	Concentration change per 0.010 Abs change
170	10 mg/L cyanuric acid	7–13 mg/L cyanuric acid	10 mg/L	0.3 mg/L cyanuric acid
			30 mg/L	0.3 mg/L cyanuric acid
			50 mg/L	0.4 mg/L cyanuric acid

Summary of method

The test for Cyanuric Acid uses the turbidimetric method. Cyanuric Acid 2 Reagent precipitates any Cyanuric Acid present and holds it in suspension. The amount of turbidity caused by the suspended particles is directly proportional to the amount of cyanuric acid present. Test results are measured at 480 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Cyanuric Acid 2 Reagent Powder Pillow	1	50/pkg	246066

Required apparatus

Description	Quantity	Unit	Catalog number
Bottle, mixing, square, with 25 mL mark	1	each	1704200
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Cyanuric Acid	25 g	712924
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, 600 g x 0.01 g, 100–240 VAC	each	2937201
Brush, test tube	each	69000
Filter, paper, for funnel filter	10/pkg	189457
Flask, volumetric, 100 mL	each	2636642
Flask, volumetric, Class A, 1000 mL	each	2636653
Funnel, filter	each	108367
Liqui-nox Solution	1 L	2088153
Pipet, TenSette, 1.0 to 10.0 mL	each	1970010
Tips for Pipet, TenSette	50/pkg	2199796
Pipet, volumetric, Class A, 3-mL	each	1451503
Pipet Filler, safety bulb	each	1465100

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Edition 7

Fluoride

DOC316.53.01041

USEPA SPADNS Method[1] **Method 8029**

0.02 to 2.00 mg/L F^- **Reagent Solution or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater; USEPA accepted for reporting for drinking and wastewater analyses (distillation required; see *Distillation* in this procedure).[2]

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater, 4500-F B & D.*

[2] Procedure is equivalent to USEPA method 340.1 for drinking water and wastewater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 145 Instrument-specific information

Instrument	Reagent solution		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

The sample and deionized water should be at the same temperature (±1 °C). Temperature adjustments may be made before or after reagent addition.
SPADNS Reagent is toxic and corrosive. Use care while handling the reagent.
SPADNS Reagent contains sodium arsenite. Final solutions will contain arsenic (D004) in sufficient concentration to be regulated as a hazardous waste for Federal RCRA. Refer to the MSDS for disposal instructions.
For best results, measure the volume of SPADNS Reagent as accurately as possible.
Do not use the Pour-Thru Cell with this test.

Collect the following items:

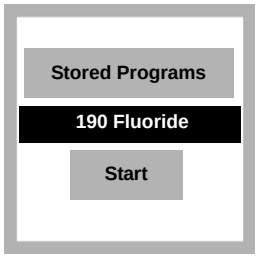
Description	Quantity
Solution Test:	
SPADNS Reagent Solution	4 mL
Deionized Water	10 mL
Pipet, volumetric, 2-mL	1
Pipet, volumetric, 10-mL	1

Collect the following items: (continued)

Description	Quantity
Pipet Filler Bulb	1
Sample cells (*Instrument-specific information*)	2
Thermometer, –10 to 110 °C	1
AccuVac Test:	
SPADNS Fluoride Reagent AccuVac® Ampuls	2
Deionized Water	40 mL
Beaker, 50-mL	1
Stopper	2

See *Consumables and replacement items* for reorder information.

SPADNS reagent solution method



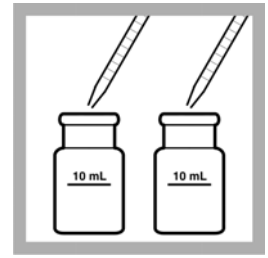
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.

2. **Prepared Sample**: Pipet 10.0 mL of sample into a dry sample cell.

3. **Blank Preparation**: Pipet 10.0 mL of deionized water into a second dry sample cell.

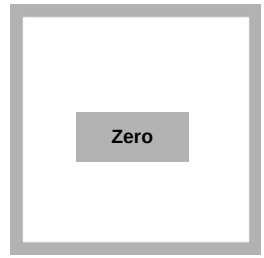


4. Carefully pipet 2.0 mL of SPADNS Reagent into each cell. Swirl to mix.

5. Start the instrument timer.

A one-minute reaction period will begin.

6. When the timer expires, insert the blank into the cell holder.



7. **ZERO** the instrument.

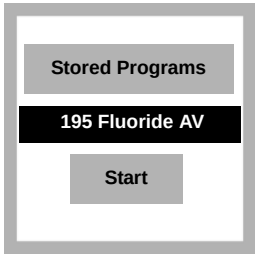
The display will show:

0.00 mg/L F^-

8. Insert the prepared sample into the cell holder.

READ the result in mg/L F^-.

SPADNS AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

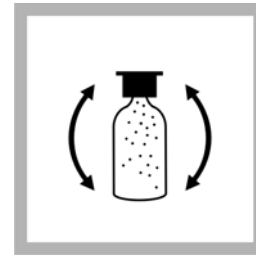
Refer to the user manual for orientation.

2. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill one SPADNS Fluoride Reagent AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

3. Blank Preparation: Pour at least 40 mL of deionized water into a second beaker.

Fill a second Ampul with deionized water. Keep the tip immersed while the Ampul fills completely.



4. Close with stopper and quickly invert both Ampuls several times to mix.

5. Start the instrument timer.

A one-minute reaction period will begin.

6. When the timer expires, wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show:

0.00 mg/L F^-

7. Insert the Ampul that contains the prepared sample into the cell holder.

READ the results in mg/L F^-.

Interferences

This test is sensitive to small amounts of interference. Glassware must be very clean (acid rinse before each use). Repeat the test with the same glassware to ensure that results are accurate.

Table 146 Interfering substances

Interfering substance	Interference level
Alkalinity (as $CaCO_3$)	At 5000 mg/L it causes a –0.1 mg/L F^- error.
Aluminum	At 0.1 mg/L it causes a –0.1 mg/L F^- error. To check for interferences from aluminum, read the concentration one minute after reagent addition, then again after 15 minutes. An appreciable increase in concentration suggests aluminum interference. Waiting 2 hours before making the final reading will eliminate the effect of up to 3.0 mg/L aluminum.
Chloride	At 7000 mg/L causes a +0.1 mg/L F^- error.
Chlorine	SPADNS Reagent contains enough arsenite to eliminate interference up to 5 mg/L chlorine. For higher chlorine levels, add one drop of Sodium Arsenite Solution[1] to 25 mL of sample for each 2 mg/L of Chlorine.
Iron, ferric	At 10 mg/L it causes a –0.1 mg/L F^- error.
Phosphate, ortho	At 16 mg/L it causes a +0.1 mg/L F^- error.
Sodium Hexametaphosphate	At 1.0 mg/L it causes a +0.1 mg/L F^- error.
Sulfate	At 200 mg/L it causes a +0.1 mg/L F^- error.

[1] See *Optional reagents and apparatus*.

Distillation

Distillation Solution Preparation:

1. Measure 60 mL of deionized water into a 250 mL glass Erlenmeyer flask.
2. With constant stirring, add 120 mL of concentrated Sulfuric Acid. ***Caution: The mixture will become very hot. Allow the solution to cool before handling.***

To eliminate most interferences, dilute the sample from the acid solution as described below:

1. Set up the distillation apparatus for general purpose distillation. Refer to the Distillation Apparatus manual for proper assembly. Use a 125-mL Erlenmeyer flask to collect the distillate.
2. Turn on the water and maintain a steady flow through the condenser.
3. Measure 100 mL of sample into the distillation flask using a 100-mL graduated cylinder. Add a magnetic stir bar and 5 glass beads.
4. Turn the stirrer power switch on. Turn the stir control to 5.
5. Using a 250-mL graduated cylinder, carefully add 150 mL of Distillation Solution into the flask.

Note: *When distilling samples with high amounts of chloride, add 5 mg of Silver Sulfate* to the sample for every mg/L of chloride in the sample.*

* See *Optional reagents and apparatus*.

6. With the thermometer in place, turn the heat control to 10. The yellow pilot lamp indicates the heater is on.
7. When the temperature reaches 180 °C or when 100 mL of distillate has been collected, turn the still off (requires about 1 hour).
8. Dilute the distillate to a volume of 100 mL, if necessary. The distillate may now be analyzed by the SPADNS or the fluoride ion-selective electrode method.

Sample collection, preservation and storage

- Samples may be stored in glass or plastic bottles for up to 28 days when cooled to 4 °C (39 °F) or lower.
- Warm samples to room temperature before analysis.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

A variety of standard solutions covering the entire range of the test is available. Use these instead of sample to verify technique.

Minor variations between lots of reagent become measurable above 1.5 mg/L. While results in this region are usable for most purposes, better accuracy may be obtained by diluting a fresh sample 1:1 with deionized water and retesting. Multiply the result by 2.

1. To adjust the calibration curve using the reading obtained with the 1.00-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
2. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
190	1.00 mg/L F^-	0.97–1.03 mg/L F^-	0.024 mg/L F^- at 1 mg/L
195	1.00 mg/L F^-	0.92–1.08 mg/L F^-	0.03 mg/L F^- at 1 mg/L

Summary of method

The SPADNS Method for fluoride determination involves the reaction of fluoride with a red zirconium-dye solution. The fluoride combines with part of the zirconium to form a colorless complex, thus bleaching the red color in an amount proportional to the fluoride concentration. This method is accepted by the EPA for NPDES and NPDWR reporting purposes when the samples have been distilled. Seawater and wastewater samples require distillation. Test results are measured at 580 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
SPADNS Reagent Solution	4 mL	500 mL	44449
OR			
SPADNS Fluoride Reagent AccuVac® Ampuls	2	25/pkg	2506025
Water, deionized	10–40 mL	4 L	27256

Required apparatus (solution)

Description	Quantity	Unit	Catalog number
Pipet Filler, safety bulb	1	each	1465100
Pipet, volumetric, Class A, 2.00-mL	1	each	1451536
Pipet, volumetric, Class A, 10.00-mL.	1	each	1451538
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Thermometer, –10 to 110 °C	1	each	187701

Required apparatus (AccuVac)

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606

Recommended standards

Description	Unit	Catalog number
Fluoride Standard Solution, 0.2-mg/L F^-	500 mL	40502
Fluoride Standard Solution, 0.5-mg/L F^-	500 mL	40505
Fluoride Standard Solution, 0.8-mg/L F^-	500 mL	40508
Fluoride Standard Solution, 1.0-mg/L F^-	1000 mL	29153
Fluoride Standard Solution, 1.0-mg/L F^-	500 mL	29149
Fluoride Standard Solution, 1.2-mg/L F^-	500 mL	40512
Fluoride Standard Solution, 1.5-mg/L F^-	500 mL	40515
Fluoride Standard Solution, 2.0-mg/L F^-	500 mL	40520
Fluoride Standard Solution, 100-mg/L F^-	500 mL	23249
Standard, Drinking Water, Mixed Parameter, Inorganic for F^-, NO_3, PO_4, SO_4	500 mL	2833049

Distillation reagents and apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 100-mL	1	each	50842
Cylinder, graduated, 250-mL	1	each	50846
Distillation Heater and Support Apparatus Set,115 VAC, 50/60 Hz	1	each	2274400
OR			

Distillation reagents and apparatus

Description	Quantity	Unit	Catalog number
Distillation Heater and Support Apparatus Set, 230 VAC, 50/60 Hz	1	each	2274402
AND			
Distillation Apparatus Set, General Purpose	1	each	2265300
Flask, Erlenmeyer, 125-mL	1	each	2089743
Glass Beads	1	100/pkg	259600
Stir Bar, magnetic	1	each	1076416
Sulfuric Acid, concentrated, ACS	1	500 mL	97949

Optional reagents and apparatus

Description	Unit	Catalog number
Silver Sulfate	113 g	33414
Sodium Arsenite Solution, 5.0 g/L	100 mL	104732
AccuVac Snapper,	each	2405200
Wipes, disposable	280/pkg	2097000

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Edition 7

Fluoride

DOC316.53.01184

USEPA[1] SPADNS 2[2] **Method 10225**

(0.02 to 2.00 mg/L F^-) **Reagent Solution or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater; USEPA accepted for reporting for drinking and wastewater analyses (distillation required; see *Distillation* in this procedure).

[1] Procedure is equivalent to USEPA method 340.1 for drinking water and wastewater analysis

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater, 4500-F B & D.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 147 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

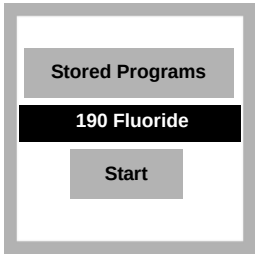
The sample and deionized water should be at the same temperature (± 1 °C). Temperature adjustments may be made before or after reagent addition.
SPADNS 2 Reagent is corrosive. Use care while handling the reagent.
For best results, measure the volume of SPADNS 2 Reagent as accurately as possible.
If the instrument displays Over Measure Range!, dilute a fresh sample with an equal volume of deionized water and repeat the test, using this solution in step 2. Multiply the result by 2.
SPADNS 2 Reagent contains a non-toxic reducing agent to prevent chlorine interference. SPADNS 2 does not contain sodium arsenite.

Collect the following items:

Description	Quantity
Solution test	
SPADNS 2 Reagent Solution	4 mL
Deionized Water	10 mL
Pipet, volumetric, 2-mL	1
Pipet, volumetric, 10-mL	1
Pipet Filler Bulb	1
Sample cells (see *Instrument-specific information*)	2
Thermometer	1
AccuVac test	
SPADNS 2 Fluoride Reagent AccuVac® Ampuls	2
Deionized Water	40 mL
Beaker, 50-mL	1

See *Consumables and replacement items* for reorder information.

SPADNS 2 reagent solution

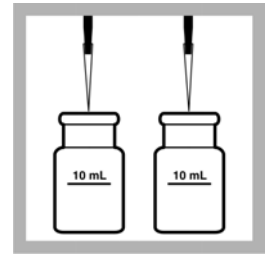


1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. Prepared Sample: Pipet 10.0 mL of sample into a dry sample cell.

3. Blank Preparation: Pipet 10.0 mL of deionized water into a second dry sample cell.



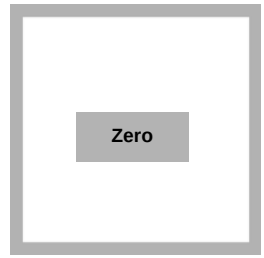
4. Carefully pipet 2.0 mL of SPADNS 2 Reagent into each cell. Swirl to mix.

SPADNS 2 reagent solution (continued)

5. Start the instrument timer.

A one-minute reaction period will begin.

6. When the timer expires, insert the blank.



7. **ZERO** the instrument.

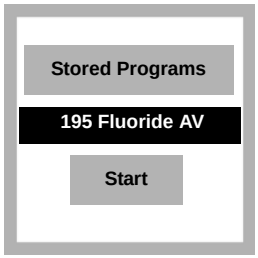
The display will show:

0.00 mg/L F^-

8. Insert the prepared sample cell.

READ the results in mg/L F^-.

SPADNS 2 AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. **Prepared Sample:** Collect at least 40 mL of sample in a 50-mL beaker.

Fill one SPADNS 2 Fluoride Reagent AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

3. **Blank Preparation:** Pour at least 40 mL of deionized water into a second beaker.

Fill a second Ampul with deionized water. Keep the tip immersed while the Ampul fills completely.

4. Quickly invert both Ampuls several times to mix.

SPADNS 2 AccuVac® Ampuls (continued)

5. Start the instrument timer.

A one-minute reaction period will begin.

6. When the timer expires, insert the blank into the cell holder.

ZERO the instrument.

The display will show:

0.00 mg/L F^-

7. Insert the prepared sample into the cell holder

READ the results in mg/L F^-.

Interferences

This test is sensitive to small amounts of interference. Glassware must be very clean (acid rinse before each use). Repeat the test with the same glassware to make sure that the results are accurate.

Table 148 Interfering substances

Interfering substance	Interference level
Alkalinity (as $CaCO_3$)	At 5000 mg/L causes a –0.1 mg/L F^- error
Aluminum	At 0.1 mg/L causes a –0.1 mg/L F^- error. To check for interferences from aluminum, read the concentration one minute after reagent addition, then again after 15 minutes. An appreciable increase in concentration suggests aluminum interference. Waiting 2 hours before making the final reading will eliminate the effect of up to 3.0 mg/L aluminum.
Chloride	At 7000 mg/L causes a +0.1 mg/L F^- error
Chlorine	SPADNS 2 Reagent contains enough non-toxic reductant to eliminate interference up to 5 mg/L chlorine. For higher chlorine levels: **1.** Dilute sample with deionized water by a factor that will lower chlorine concentration to below 5 mg/L. **2.** Perform the SPADNS 2 reagent solution or AccuVac procedure. **3.** Multiply results by the dilution factor to obtain mg/L Fluoride.
Iron, ferric	At 10 mg/L causes a –0.1 mg/L F^- error
Phosphate, ortho	At 16 mg/L causes a +0.1 mg/L F^- error
Sodium Hexametaphosphate	At 1.0 mg/L causes a +0.1 mg/L F^- error
Sulfate	At 200 mg/L causes a +0.1 mg/L F^- error

Distillation

Distillation Solution Preparation:

1. Measure 60 mL of deionized water into a 250 mL glass Erlenmeyer flask.
2. With constant stirring, add 120 mL of concentrated Sulfuric Acid. ***Caution: The mixture will become very hot. Allow the solution to cool before handling.***

To eliminate most interferences, dilute the sample from the acid solution as described below:

1. Set up the distillation apparatus for general purpose distillation. Refer to the Distillation Apparatus manual for proper assembly. Use a 125-mL Erlenmeyer flask to collect the distillate.
2. Turn on the water and maintain a steady flow through the condenser.
3. Measure 100 mL of sample into the distillation flask using a 100-mL graduated cylinder. Add a magnetic stir bar and 5 glass beads.
4. Turn the stirrer power switch on. Turn the stir control to 5.
5. Using a 250-mL graduated cylinder, carefully add 150 mL of Distillation Solution into the flask.

Note: *When distilling samples with high amounts of chloride, add 5 mg of Silver Sulfate to the sample for every mg/L of chloride in the sample.*

6. With the thermometer in place, turn the heat control to 10. The yellow pilot lamp indicates the heater is on.
7. When the temperature reaches 180 °C or when 100 mL of distillate has been collected, turn the still off (requires about 1 hour).
8. Dilute the distillate to a volume of 100 mL, if necessary. The distillate may now be analyzed by the SPADNS, SPADNS 2 or the fluoride ion-selective electrode method.

Sample collection, preservation and storage

- Samples may be stored in glass or plastic bottles for at least seven days when cooled to 4 °C (39 °F) or lower.
- Warm samples to room temperature before analysis.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

A variety of standard solutions for the entire range of the test is available. Use standard solutions instead of sample to verify the technique.

Minor variations between lots of reagent become measurable above 1.5 mg/L. While results in this region are usable for most purposes, better accuracy may be obtained with steps 1–3.

1. Dilute a fresh sample 1:1 with deionized water.
2. Perform the test again
3. Multiply the result by 2.
4. To adjust the calibration curve with the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.

5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
190	1.00 mg/L F^-	0.97–1.03 mg/L F^-	0.024 mg/L F^- at 1 mg/L
195	1.00 mg/L F^-	0.92–1.08 mg/L F^-	0.03 mg/L F^- at 1 mg/L

Safety

Follow good safety habits and laboratory techniques throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used.

Pollution prevention and waste management

SPADNS 2 Reagent does not contain sodium arsenite. Instead, it contains a non-toxic species to prevent chlorine interference. Dispose of all waste safely in accordance with local and federal guidelines.

Summary of method

The SPADNS 2 Method for fluoride determination involves the reaction of fluoride with a red zirconium-dye solution. The fluoride combines with part of the zirconium to form a colorless complex that bleaches the red color in an amount proportional to the fluoride concentration. This method is equivalent to the EPA method for NPDES and NPDWR reporting purposes when the samples have been distilled. Seawater and wastewater samples require distillation. Test results are measured at 580 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
SPADNS 2 Reagent Solution	4 mL	500 mL	2947549
OR			
SPADNS 2 Fluoride Reagent AccuVac® Ampuls	2	25/pkg	2527025
Water, deionized	10 mL	4 L	27256

Required apparatus (solution)

Description	Quantity	Unit	Catalog number
Pipet Filler, safety bulb	1	each	1465100
Pipet, volumetric, Class A, 2.00-mL	1	each	1451536
Pipet, volumetric, Class A, 10.00-mL.	1	each	1451538
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Thermometer	1	each	2635700

Required apparatus (AccuVac)

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606

Recommended standards

Description	Unit	Catalog number
Fluoride Standard Solution, 0.2-mg/L F^-	500 mL	40502
Fluoride Standard Solution, 0.5-mg/L F^-	500 mL	40505
Fluoride Standard Solution, 0.8-mg/L F^-	500 mL	40508
Fluoride Standard Solution, 1.0-mg/L F^-	1000 mL	29153
Fluoride Standard Solution, 1.0-mg/L F^-	500 mL	29149
Fluoride Standard Solution, 1.2-mg/L F^-	500 mL	40512
Fluoride Standard Solution, 1.5-mg/L F^-	500 mL	40515
Fluoride Standard Solution, 2.0-mg/L F^-	500 mL	40520
Fluoride Standard Solution, 100-mg/L F^-	500 mL	23249
Standard, Drinking Water, Mixed Parameter, Inorganic for F^-, NO_3, PO_4, SO_4	500 mL	2833049

Distillation reagents and apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 100-mL	1	each	50842
Cylinder, graduated, 250-mL	1	each	50846
Distillation Heater and Support Apparatus Set,115 VAC, 50/60 Hz	1	each	2274400
AND			
Distillation Heater and Support Apparatus Set,230 VAC, 50/60 Hz	1	each	2274402
OR			
Distillation Apparatus Set, General Purpose	1	each	2265300
Flask, Erlenmeyer, 125-mL	1	each	2089743
Flask, Erlenmeyer, 250 mL, Glass	1	each	50546
Glass Beads	1	100/pkg	259600
Stir Bar, magnetic	1	each	1076416
Sulfuric Acid, ACS	1	500 mL	97949

Optional reagents and apparatus

Description	Unit	Catalog number
Silver Sulfate	113 g	33414
Balance Analytical 80 g x 0.1 mg 100–240 V	each	2936701
Weighing papers	500/pkg	1473800

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Edition 7

Formaldehyde

DOC316.53.01042

MBTH Method[1] **Method 8110**

3 to 500 µg/L CH_2O **Powder Pillows**

Scope and Application: For water.

[1] Adapted from Matthews, T.G. and Howell, T.C., *Journal of the Air Pollution Control Association*, 31 (11) 1181-1184 (1981).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 149 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Analyze samples immediately. Do not preserve for later analysis.
Wash glassware with Chromic Acid Cleaning Solution[1] to remove trace contaminants.
Time and temperature are very important to this test. The sample should be 25 ± 1 °C and the times specified in the procedure steps must be followed precisely. A temperature-controlled water bath is recommended for best accuracy.
Obtain formaldehyde-free water by distilling water from alkaline permanganate (4 g sodium hydroxide[1], 2 g potassium permanganate per 500 mL of water). Discard the first 50–100 mL of distillate.
The Pour-thru cell cannot be used for this test.

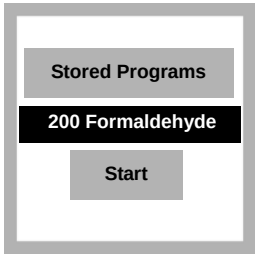
[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
Developing Solution for LR Formaldehyde	5 mL
MBTH Powder Pillows	2
Cylinder, graduated mixing, 50-mL	2
Pipet, serological, 5-mL	1
Pipet Filler	1
Sample Cells (*Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

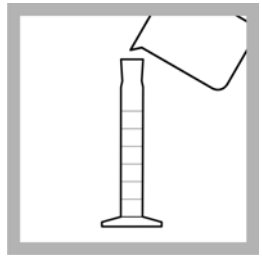
MBTH method for powder pillows



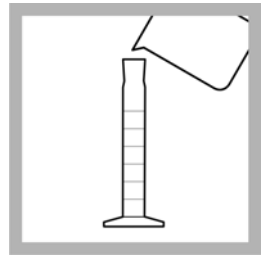
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

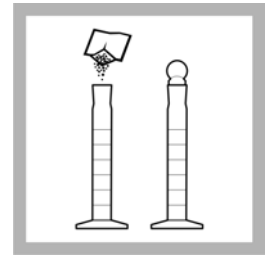
Refer to the user manual for orientation.



2. Prepared Sample: Accurately measure 25 mL of sample in a 50-mL mixing cylinder.



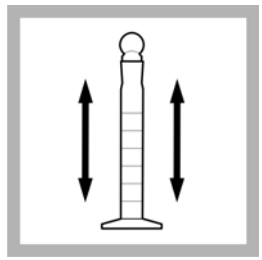
3. Blank Preparation: Accurately measure 25 mL of formaldehyde-free water in a second 50-mL mixing cylinder.



4. Add the contents of one MBTH powder pillow to the blank. Insert the cylinder stopper.

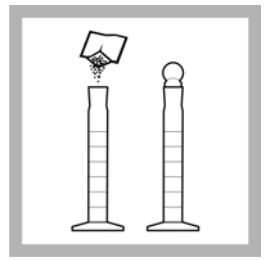
5. Start the instrument timer. A 17-minute reaction period will begin.

Proceed with step 6 immediately after the timer starts.

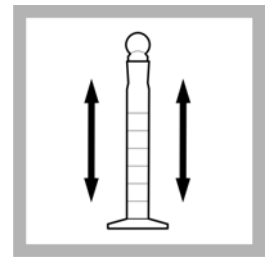


6. Immediately after the reaction period starts, shake the blank sample vigorously for 20 seconds.

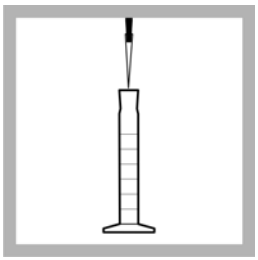
Do not wait for the timer to expire.



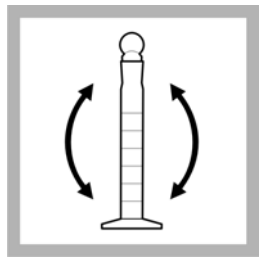
7. Add the contents of one MBTH powder pillow to the prepared sample when the timer shows **15:00**. Insert the cylinder stopper.



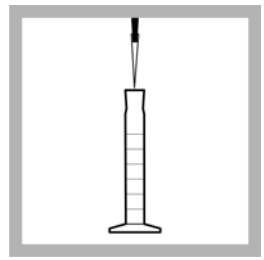
8. Shake vigorously for 20 seconds.



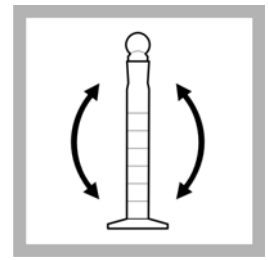
9. Add 2.5 mL of Developing Solution for Low Range Formaldehyde to the blank when the timer shows **12:00**.



10. Insert the cylinder stopper and invert to mix.



11. Add 2.5 mL of Developing Solution for Low Range Formaldehyde to the prepared sample when the timer shows **10:00**.

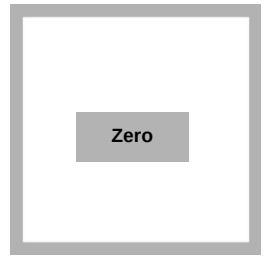


12. Insert the cylinder stopper and invert to mix.

MBTH method for powder pillows (continued)

13. Just before the timer shows **2:00**, pour at least 10 mL of the blank into the sample cell. Pour the solution slowly to avoid bubble formation on the cell walls. If bubbles form, swirl to dislodge them.

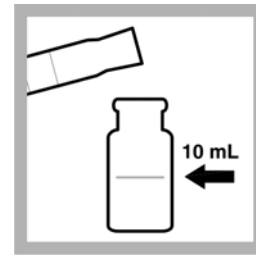
14. Immediately wipe the blank and insert it into the cell holder.



15. When the timer shows **2:00**, **ZERO** the instrument.

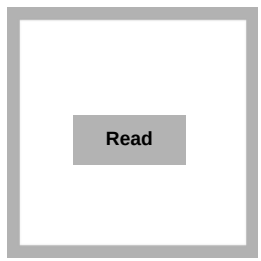
The display will show:

0 µg/L CH_2O



16. Pour at least 10 mL of the prepared sample into a sample cell.

17. Wipe the cell and insert it into the cell holder.



18. When the timer expires, **READ** the results in µg/L CH_2O.

Interferences

Table 150 Interfering substances

Interfering substance	Interference level	Interfering substance	Interference level
Acetate	Greater than 1000 mg/L	Iron (Fe^{3+})	Greater than 12 mg/L
Aldehydes (other)	Positive interference at all levels	Lead	Greater than 100 mg/L
Ammonium (as N)	Greater than 10 mg/L	Manganese	Greater than 500 mg/L
Aniline	Greater than 10 mg/L	Mercury	Greater than 70 mg/L
Bicarbonate	Greater than 1000 mg/L	Morpholine	Greater than 0.36 mg/L
Calcium	Greater than 3500 mg/L	Nitrate	Greater than 1000 mg/L
Carbonate	Greater than 500 mg/L	Nitrite	Greater than 8 mg/L
Chloride	Greater than 5000 mg/L	Phenol	Greater than 1050 mg/L
Copper	Greater than 1.6 mg/L	Phosphate	Greater than 200 mg/L
Cyclohexylamine	Greater than 250 mg/L	Silica	Greater than 40 mg/L

Table 150 Interfering substances (continued)

Interfering substance	Interference level	Interfering substance	Interference level
Ethanolamine	Greater than 33 mg/L	Sulfate	Greater than 10,000 mg/L
Ethylenediamine	Greater than 1.5 mg/L	Urea	Greater than 1000 mg/L
Glucose	Greater than 1000 mg/L	Zinc	Greater than 1000 mg/L
Glycine	Greater than 1000 mg/L		

Accuracy check

Required for accuracy check:

- Formaldehyde Voluette® Ampule Standard, 4000-mg/L CH_2O
- TenSette Pipet and Pipet Tips
- Flask, 100-mL volumetric Class A
- Mixing cylinders, 50-mL (3)

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **Options>More>Standard Additions** from the instrument menu.
3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a Formaldehyde Voluette® Ampule Standard, 4000-mg/L CH_2O.
5. Use a TenSette® Pipet to add 0.2 mL of the standard to a 100-mL volumetric Class A flask. Dilute to volume with formaldehyde-free water and mix well. Prepare daily. This is an 8000-µg/L (8-mg/L) formaldehyde standard.
6. Prepare three sample spikes. Fill three 50-mL mixing cylinders* with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of 8000-µg/L standard, respectively, to each sample and mix thoroughly.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard addition. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery

Standard solution method

Prepare a 320-µg/L Formaldehyde Standard Solution by pipetting 1.0 mL of the 8000-µg/L solution into a 50-mL mixing cylinder. Dilute to 25.0 mL with formaldehyde-free water. Run the test directly on this sample.

* See *Optional reagents and apparatus*.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
200	320 µg/L CH_2O	312–328 µg/L CH_2O	3 µg/L CH_2O

Summary of method

Formaldehyde reacts with MBTH (3-methyl-2-benzothiazoline hydrazone) and a developing solution to form a blue color in proportion to the formaldehyde concentration. Test results are measured at 630 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Formaldehyde Reagent Set (100 tests), includes:	—	—	2257700
Developing Solution for LR Formaldehyde	5 mL	500 mL	2257249
MBTH Powder Pillows	2	100/pkg	2257169

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated mixing, 50-mL	2	each	189641
Pipet, serological, 5-mL	1	each	53237
Pipet Filler, safety bulb	1	each	1465100
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Formaldehyde Standard Solution, 10-mL Voluette® Ampule, 4000-mg/L	16/pkg	2257310

Optional reagents and apparatus

Description	Unit	Catalog number
Chromic Acid Cleaning Solution	500 mL	123349
Potassium Permanganate	454 g	16801H
Sodium Hydroxide ACS	500 g	18734
Flask, volumetric, Class A, 100 mL	each	1457442
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, Tensette	50/pkg	2185696
Ampule Breaker	each	2196800
Wipes, disposable	280/pkg	2097000

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Edition 7

Calcium and Magnesium; Calmagite Colorimetric Method

Method 8030

0.05 to 4.00 mg/L Ca and Mg as $CaCO_3$

Scope and Application: For water, wastewater and seawater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 151 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

For the most accurate magnesium test results, keep the sample temperature between 21–29 °C (70–84 °F).
The test will detect any calcium or magnesium contamination in the mixing cylinder, measuring droppers or sample cells. To test cleanliness, repeat the test until results are consistent.
Total hardness in mg/L equals mg/L Ca as $CaCO_3$ plus mg/L Mg as $CaCO_3$.
Remaining traces of EDTA or EGTA from previous tests will give erroneous results. Rinse sample cells thoroughly before using.

Collect the following items:

Description	Quantity
Alkali Solution for Calcium and Magnesium test	1 mL
Calcium and Magnesium Indicator Solution	1 mL
EDTA Solution, 1 M	1 drop
EGTA Solution	1 drop
Cylinder, 100-mL, graduated mixing	1
Dropper, measuring, 0.5 and 1.0 mL	2
Sample Cells (*Instrument-specific information*)	3

Collect the following items:

Description	Quantity

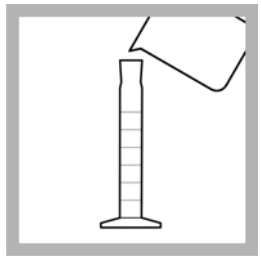
See *Consumables and replacement items* for reorder information.

Calmagite

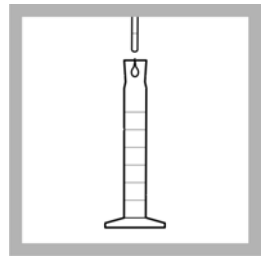
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

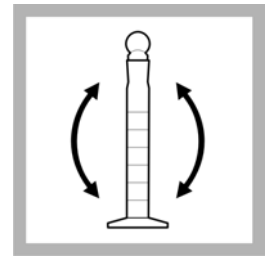
Refer to the user manual for orientation.



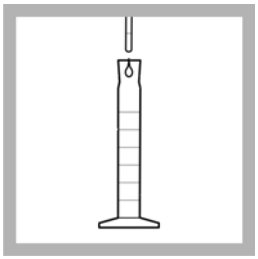
2. Pour 100 mL of sample into a 100-mL graduated mixing cylinder.



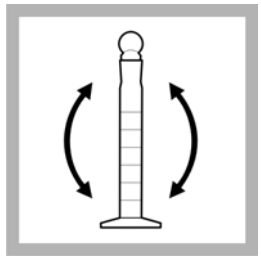
3. Use a 1.0 mL measuring dropper to add 1.0 mL of Calcium and Magnesium Indicator solution.



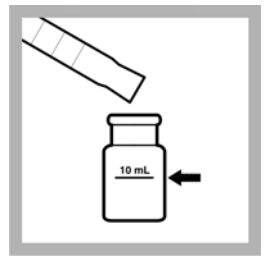
4. Stopper the cylinder and invert it several times.



5. Add 1.0 mL of Alkali Solution for Calcium and Magnesium Test using a 1.0 mL measuring dropper.



6. Stopper the cylinder and invert it several times.



7. Pour 10 mL of the solution into each of three sample cells.

8. Blank Preparation: Add one drop of 1 M EDTA Solution to the **first** cell (blank).

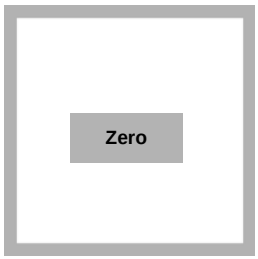
Calmagite (continued)

9. Swirl to mix.

10. Magnesium Sample: Add one drop of EGTA Solution to the **second** cell.

11. Swirl to mix.

12. Insert the **blank** (first cell) into the cell holder.

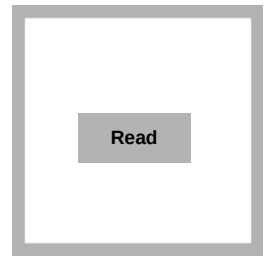


13. ZERO the instrument.

The display will show:

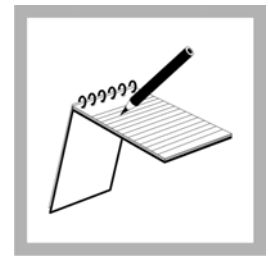
0.00 mg/L Mg $CaCO_3$

14. Insert the **magnesium sample** (second cell) into the cell holder.



15. READ the results in mg/L magnesium as calcium carbonate.

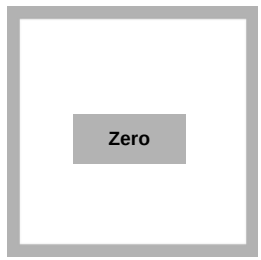
This value is the amount of magnesium in the sample expressed as $CaCO_3$.



16. Do not remove the cell from the instrument. Record or store the magnesium results before proceeding with step 17.

17. EXIT the magnesium program

Select the calcium test.



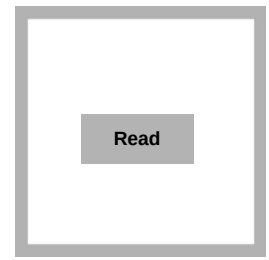
18. ZERO the instrument.

The display will show:

0.00 mg/L Ca $CaCO_3$

Remove the second cell.

19. Calcium Sample: Insert the **third** cell into the cell holder.



20. READ the results in mg/L calcium as calcium carbonate.

This value is the amount of calcium in the sample expressed as $CaCO_3$.

Interferences

Table 152 Interfering substances and levels

Interfering substance	Interference levels and treatments
Chromium (Cr^{3+})	Above 0.25 mg/L
Copper (Cu^{2+})	Above 0.75 mg/L
EDTA	Above 0.2 mg/L as $CaCO_3$
EDTA or EGTA	Traces remaining in sample cells from previous tests will give erroneous results. Rinse cells thoroughly before using.
Iron (Fe^{2+})	Above 1.4 mg/L
Iron (Fe^{3+})	Above 2.0 mg/L
Manganese (Mn^{2+})	Above 0.20 mg/L
Zinc (Zn^{2+})	Above 0.050 mg/L
Ca >1.0 mg/L; Mg >0.25 mg/L	For the most accurate calcium test result, rerun the test on a diluted sample if the calcium is over 1.0 and the magnesium is over 0.25 mg/L as $CaCO_3$. No retesting is needed if either is below those respective concentrations.

Sample collection, preservation and storage

- Collect samples in acid-washed plastic bottles.
- Adjust the sample pH to 2 or less with Nitric Acid (about 5 mL per liter).
- Cool samples to 4 °C. Preserved samples can be stored up to six months.
- Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution*.
- Correct the test results for volume additions.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
220	2.00 mg/L Ca	1.90–2.10 mg/L Ca	0.05 mg/L Ca
225	2.00 mg/L Mg	1.92–2.08 mg/L Mg	0.02 mg/L Mg

Summary of method

The colorimetric method for measuring hardness supplements the conventional titrimetric method because the colorimetric method can measure very low levels of calcium and magnesium. Also, some metals (those listed in Table 152) that interfere in the titrimetric method may be inconsequential when diluting the sample to bring it within the range of this test. The indicator dye is calmagite, which forms a purplish-blue color in a strongly alkaline solution and changes to red when it reacts with free calcium or magnesium. Calcium and magnesium determinations are made by chelating calcium with EGTA to destroy any red color due to calcium and then chelating the calcium and magnesium with EDTA to destroy the red color due to both calcium and magnesium.

* See Optional Reagents and Apparatus on page 4

By measuring the red color in the different states, calcium and magnesium concentrations are determined. Test results are measured at 522 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hardness Reagent Set (100 tests), includes:	—	—	2319900
Alkali Solution for Calcium and Magnesium test	1 mL	100 mL MDB	2241732
Calcium and Magnesium Indicator Solution	1 mL	100 mL MDB	2241832
EDTA Solution, 1 M	1 drop	50 mL SCDB	2241926
EGTA Solution	1 drop	50 mL SCDB	2229726

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, 100 mL, graduated mixing	1	each	189642
Dropper, measuring, 0.5 and 1.0 mL	2	20/pkg	2124720
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional reagents and apparatus

Description	Unit	Catalog number
Nitric Acid, ACS	500 mL	15249
Sodium Hydroxide Standard Solution, 5.0 N	10 mL MDB	245032
pH Paper, 0–14	100/pkg	2601300

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Edition 7

Hardness

DOC316.53.01044

Calcium and Magnesium;
Chlorophosphonazo Colorimetric Method

Method 8374

(8 to 1000 µg/L Ca and Mg as $CaCO_3$)

Solution Pillow

Scope and Application: For boiler and ultra-pure water.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 153 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

For the most accurate magnesium test results, keep the sample temperature between 21–29 °C (70–84 °F).
The test will detect any calcium or magnesium contamination in the measuring droppers or sample cells. To test cleanliness, repeat the test until results are consistent.
If the sample concentration is greater than 750 µg/L, a 1:1 dilution of the sample is recommended for greatest accuracy. Use ultra-pure (aldehyde-free) water for the dilution. Repeat the analysis on the diluted sample and multiply the resulting concentration by two.Alternate forms should only be used when the sample is known to contain only Mg or Ca. This method does not distinguish between the two forms.
Alternate forms should only be used when the sample is known to contain only Mg or Ca. This method does not distinguish between the two forms.
One mL of Chlorphosphonazo Solution may be used instead of the solution pillow in step 4.
Use dedicated plasticware for this analysis.
Use a bottle-top dispenser to dispense the reagent
Total hardness in mg/L equals mg/L Ca as $CaCO_3$ plus mg/L Mg as $CaCO_3$.

Collect the following items:

Description	Quantity
ULR Hardness Reagent Set	1

Collect the following items:

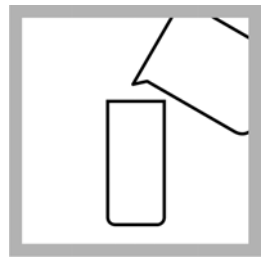
Description	Quantity
Chlorophosphonazo Indicator Solution Pillow	1
CDTA Solution	1 drop
Shears for opening powder pillow	1
Sample Cells (2410212)	1

See *Consumables and replacement items* for reorder information.

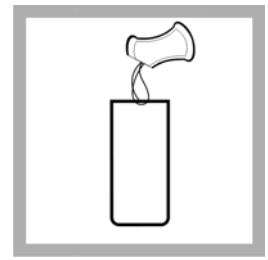
Chlorophosphonazo method

1. Select the test.

2. Rinse a plastic sample cell and the cap three times with the water to be tested. Do not allow the underside of the cap to come in contact with surfaces that may contaminate it.



3. Fill the plastic sample cell to the 25-mL mark with sample.

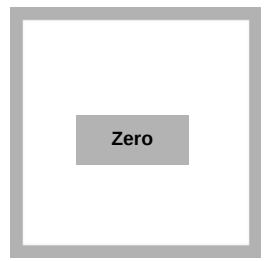


4. Add the contents of one Chlorophosphonazo Solution Pillow to the sample cell.

A small amount of solution may remain in the pillow. This will not affect results.

5. Cap the cell and swirl to mix.

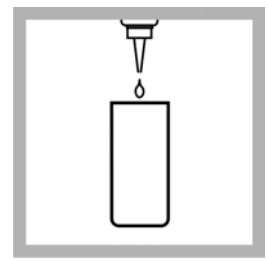
6. Insert the cell into the cell holder.



7. **ZERO** the instrument.

The display will show:

0 µg/L $CaCO_3$



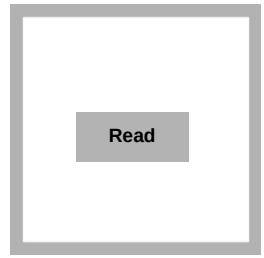
8. Remove the cell from the holder. Add one drop of CDTA Reagent for Ultra Low Range Hardness.

Complete steps 10-11 within 1-2 minutes.

Chlorophosphonazo method (continued)

9. Cap the cell and swirl to mix.

10. Insert the cell into the holder.



11. **READ** the results in µg/L $CaCO_3$.

Interferences

Interference studies were conducted at various hardness levels between 0 and 500 µg/L as $CaCO_3$ (*Interfering substances and levels*). Various cations and anions were evaluated at levels in the range appropriate for ultra pure water applications. An ion is said to interfere when the resulting concentration is changed by ± 10%.

Table 154 Interfering substances and levels

Interfering Substance	Interference Levels and Treatments
Aluminum	Negative interference above 150 µg/L
Ammonium	No interference at or below 1000 µg/L
Copper	Positive interference above 250 µg/L
Formaldehyde	No interference at or below 47,000 µg/L
Nitrate	Positive interference above 250 µg/L
Potassium	No interference at or below 1000 µg/L
Silicon	Positive interference above 1000 µg/L
Sodium	Negative interference above 79,000 µg/L

Sample collection, preservation and storage

- Do not use glass containers.
- Collect samples in clean plastic containers, preferably with screw-type closures.
- Rinse containers several times with the water to be analyzed before collecting the final sample.
- Seal to avoid contamination during transport.
- Analyze as soon as possible.

Accuracy check

Required for accuracy check:

- Calcium Chloride Standard Solution, 50 mg/L (50,000 µg/L) as $CaCO_3$
- TenSette Pipet and Pipet Tips

Standard additions method (sample spike)

1. Prepare a 20,000 µg/L (20 mg/L) $CaCO_3$ standard by pipetting 20 mL of 50 mg/L $CaCO_3$ standard solution into a 50 mL plastic volumetric flask. Dilute the solution with 50 mL of ultra-pure water.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select Options>More>Standard Additions from the instrument menu.
4. Accept the default sample volume (25 mL).
5. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the prepared 20,000 µg/L standard, respectively to three 25-mL samples and mix each thoroughly.
6. Follow the test procedure for each of the spiked samples.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Use a 0.50 mg/L $CaCO_3$ (500-µg/L as $CaCO_3$) Calcium Chloride Standard Solution in place of the sample.
2. To adjust the calibration curve using the reading obtained with the 0.50-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
278	500 µg/L	478–522 mg/L	8 µg/L Ca

Summary of method

Calcium and magnesium combine equivalently with the Chlorophosphonazo III indicator to form a colored complex which absorbs light very strongly at 669 nm. One drop of the CDTA reagent breaks up this complex and the resultant decrease in color is proportional to the amount of calcium and magnesium in the sample (as $CaCO_3$). Test results are measured at 669 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
ULR Hardness Reagent Set (100 tests), includes:	—	—	2603100
(1) Chlorophosphonazo Indicator Solution Pillows	1	100/pkg	2589599
(1) CDTA Solution	1 drop	10 mL SCDB	2589636
(1) Sample cell, 1-inch square, molded	1	each	2410201
(1) Sample cell cap	1	each	2410202

Required reagents

Description	Quantity/Test	Unit	Catalog number
OR			
ULR Hardness Reagent Set (500 tests), includes:	—	—	2603101
(1) Chlorophosphonazo Indicator Solution	1 mL	500 mL	2589549
(2) CDTA Solution	1 drop	10 mL SCDB	2589636
(1) Sample cell, 1-inch square, molded	1	each	2410201
(1) Sample cell cap	1	each	2410202
Chlorophosphonazo Indicator Solution Pillows	1	100/pkg	2589599
CDTA Solution	1 drop	10 mL SCDB	2589636

Required apparatus

Description	Quantity	Unit	Catalog number
Shears	1	each	2369400

Recommended standards and apparatus

Description	Unit	Catalog number
Calcium Chloride Standard Solution, 50 mg/L as $CaCO_3$	946 mL	2127716
Calcium Chloride Standard Solution, 500 µg/L as $CaCO_3$	946 mL	2058016
Chlorophosphonazo Indicator Solution	500 mL	2589549

Optional reagents and apparatus

Description	Unit	Catalog number
Dispenser, 1.0-mL, Repipet Jr.	each	2111302
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet, volumetric, Class A, 20 mL	each	1451520
Flask, volumetric, polypropylene, 50 mL	each	1406041
Pipet filler, Safety Bulb	each	1465100
Sample cell, 25-mL plastic with cap	2	12/pkg

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Edition 7

Hardness, Total

DOC316.53.01045

Calcium and Magnesium;
Chlorophosphonazo Rapid Liquid Method

Method 8374

ULR (4 to 1000 µg/L Ca and Mg as $CaCO_3$)

Pour-Thru Cell

Scope and Application: For boiler and ultra pure water.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 155 Instrument-specific information

Instrument	Pour-thru Kit	Cell orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

Pre-clean the Pour-Thru Cell and all labware as specified in *Treating analysis labware*.
For the most accurate magnesium test results, keep the sample temperature between 21–29 °C (70–84 °F).
The test will detect any calcium or magnesium contamination in the mixing cylinder, measuring droppers, or sample cells. To test cleanliness, repeat the test until results are consistent.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
If the sample concentration is greater than 750 µg/L, a 1:1 dilution of the sample is recommended for greatest accuracy. Use ultra-pure (aldehyde-free) water for the dilution. Repeat the analysis on the diluted sample and multiply the resulting concentration by two.
Alternate forms should only be used when the sample is known to contain only Mg or Ca. This method does not distinguish between the two forms.
Refer to the instrument User Manual for Pour-Thru cell and module assembly and installation.
Use dedicated plasticware for this analysis.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the DR 2800 or DR 2700 cell compartment with the protective cover during measurements.
Total hardness in mg/L equals mg/L Ca as $CaCO_3$ plus mg/L Mg as $CaCO_3$.
Ensure the pour-thru cell is completely seated in the sample cell compartment.

Collect the following items:

Description	Quantity
Chlorophosphonazo Indicator Solution	2 mL
CDTA Reagent for Ultra Low Range Hardness	1 drop
Water, Ultra-pure	Varies
Cylinder, graduated, 50-mL, poly	1
Dispenser, Adjustable Volume	1
Flask, Erlenmeyer, PMP w/cap, 125-mL	1
Pour-Thru Cell Module (see *Instrument-specific information* table)	1

See *Consumables and replacement items* for reorder information.

Chlorophosphonazo rapid liquid method

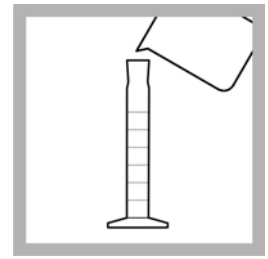
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

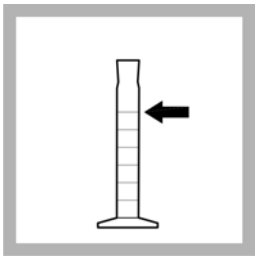
2. Flush the Pour-Thru Cell with 50 mL of ultra-pure water.

3. Fill a clean, 125-mL plastic Erlenmeyer flask to overflowing with sample.

Collect sample directly in the flask if possible.



4. Rinse a clean, 50-mL plastic graduated cylinder three times with sample.



5. Fill the rinsed cylinder to the 50-mL mark with sample from the flask. Discard remaining contents of the flask.

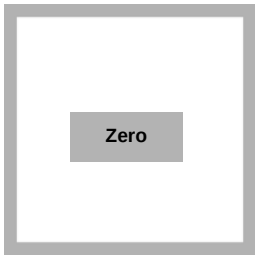
6. Pour the contents of the 50-mL cylinder back into the flask.

7. Add 2.0 mL of Chlorophosphonazo Reagent to the sample with the Dispenser. Swirl to mix.

8. Pour approximately half (25 mL) of the sample into the Pour-Thru Cell.

Use a clean, dry, plastic 25-mL graduated cylinder to measure the sample.

Chlorophosphonazo rapid liquid method (continued)



9. After the flow stops, **ZERO** the instrument.

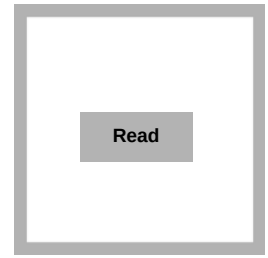
The display will show:

0 µg/L $CaCO_3$

10. Add one drop of CDTA Reagent for Ultra Low Range Hardness to the remaining sample in the flask. Swirl to mix.

Complete steps 11 and 12 within one to two minutes.

11. Pour the remaining sample into the Pour-Thru Cell.



12. **READ** the results in µg/L $CaCO_3$.

13. Using a wash bottle, rinse the Pour-Thru Cell with at least 75 mL of ultra-pure water immediately after use.

Rinse the flask with ultra-pure water. Cap when finished.

Interferences

Table 156 Interfering substances

Interfering substance	Interference level
Aluminum	Negative interference above 150 µg/L
Ammonium	No interference at or below 1000 µg/L
Copper	Positive interference above 250 µg/L
Formaldehyde	No interference at or below 47,000 µg/L
Potassium	No interference at or below 1000 µg/L
Silicon	Positive interference above 1000 µg/L
Sodium	Negative interference above 79,000 µg/L

Treating analysis labware

Clean all containers used in this test thoroughly to remove any traces of calcium or magnesium. If possible, use plastic containers for all analysis and storage. Clean containers by normal means, then rinse with ultra-pure (aldehyde-free) water. Fill and soak for 10 minutes with a 1:25 dilution of Chlorophosphonazo Reagent in ultra-pure water. Rinse well with ultra-pure water. Keep containers tightly closed and dedicate them for ULR Hardness only. If containers are rinsed and capped after each use, only occasional soaking is necessary. Fill the Pour-Thru cell with this same mixture of chlorophosphonazo and water and let stand for several minutes. Rinse with ultra-pure water.

Avoid contamination of the Chlorophosphonazo Reagent bottle when placing the Repipet dispenser on the bottle. Rinse the inlet tubing and inside of the dispenser cap with copious amounts of ultra-pure water using a wash bottle. Place the inlet tubing into a beaker of ultra-pure water and depress the plunger 10–15 times to rinse the inside of the dispenser. (For best results, pour a small amount of reagent into the beaker of rinse water.) Remove the dispenser from the water and depress the plunger until all of the water has been expelled.
Shake off any excess water on the dispenser, place the dispenser on the bottle and tighten.

Sample collection, preservation and storage

- Do not use glass containers.
- Collect samples in clean plastic containers, preferably with screw-type closures.
- Rinse containers several times with the water to be analyzed before collecting the final sample.
- Seal to avoid contamination during transport.
- Analyze as soon as possible.

Accuracy check

Required for accuracy check:

- Calcium Chloride Standard Solution, 50 mg/L (50,000 µg/L) as $CaCO_3$
- TenSette® Pipet and Pipet Tips

Standard additions method (sample spike)

1. Prepare a 20,000 µg/L (20 mg/L) $CaCO_3$ standard by pipetting 20 mL of 50 mg/L $CaCO_3$ standard solution into a 50 mL plastic volumetric flask. Dilute the solution with 50 mL of ultra-pure water.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select Options>More>Standard Additions from the instrument menu.

4. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. Accept the default values then read the unspiked sample measurement. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
5. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL, and 0.6 mL of the prepared 20,000 µg/L standard to three 50 mL portions of fresh sample.
6. Follow the test procedure for each of the spiked samples, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Using a 0.50 mg/L (500 µg/L as $CaCO_3$) Calcium Chloride Standard Solution, perform the procedure using the standard in place of the sample.
2. To adjust the calibration curve using the reading obtained with the 0.50-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
227	0.500 mg/L Cr^{6+}	495–505 µg/L	8µg/L Ca

Summary of method

Calcium and magnesium combine equivalently with the Chlorophosphonazo Indicator to form a colored complex which absorbs light very strongly at 669 nm. One drop of the CDTA reagent breaks up this complex, and the resultant decrease in color is proportional to the amount of calcium and magnesium (as $CaCO_3$) in the sample. Test results are measured at 669 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Chlorophosphonazo Indicator Solution	2 mL	500 mL	2589549
CDTA Reagent for Ultra Low Range Hardness	1 drop	10 mL SCDB	2589636

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 50 mL, poly	1	each	108141
Dispenser, variable-volume	1	each	2563137
Flask, Erlenmeyer, PMP w/cap, 125 mL	1	each	2089843

Recommended standards

Description	Unit	Catalog number
Calcium Standard Solution, 50 mg/L as $CaCO_3$	946 mL	2127716
Calcium Standard Solution, 0.50 mg/L as $CaCO_3$	946 mL	2058016
Water, ultra-pure (aldehyde-free)	500 mL	2594649

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, TenSette® 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet, volumetric, Class A, 20 mL	each	1451520
Pipet filler, Safety bulb	each	1465100
Flask, volumetric, polypropylene, 50 mL	each	1406041

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Edition 7

Hardness, Calcium

DOC316.53.01175

Titration Method using EDTA

Method 8204

10 to 4000 mg/L as $CaCO_3$

Digital Titrator

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

Magnesium is not included in the results but must be present for a sharp end point. If magnesium is not present, add one to two drops of Magnesium Standard Solution, 10-g/L as $CaCO_3$ to the sample before the test is started.
One German degree of hardness (G.d.h.) = 17.9 mg/L hardness as $CaCO_3$
mg/L Ca = Ca hardness in mg/L as $CaCO_3$ x 0.40
A 0.1-g scoop of CalVer® 2 Calcium Indicator Powder can be used in place of the CalVer 2 Calcium Indicator Powder Pillow.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

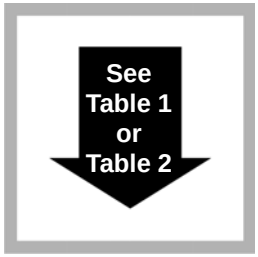
[1] See *Optional reagents and apparatus*.

Collect the following items:

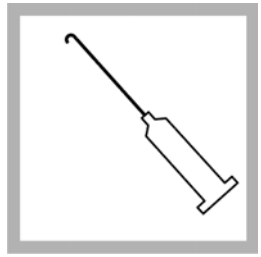
Description	Quantity
CalVer 2 Calcium Indicator Powder Pillow	1 pillow
Potassium Hydroxide Standard Solution, 8 N	1 bottle
EDTA titration cartridge (see *Range-specific information—mg/L* (mg/L) or *Range-specific information—G.d.h.* (German degrees of hardness))	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Hardness, Calcium

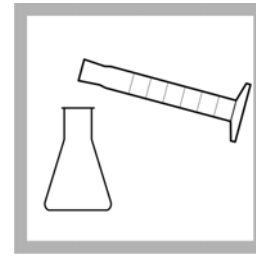


1. Select a sample volume and titration cartridge from the *Range-specific information—mg/L* table or the *Range-specific information—G.d.h.* table.



2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

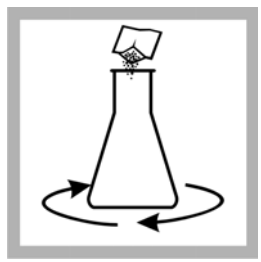
3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.



4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information—mg/L* or the *Range-specific information—G.d.h.* table.

Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.

5. If the sample volume is 100 mL, add 2 mL of 8 N Potassium Hydroxide Standard Solution. If the sample volume is 50 mL or less, add 1 mL of 8 N Potassium Hydroxide Standard Solution. Swirl to mix.



6. Add the contents of one CalVer 2 Calcium Indicator Powder Pillow. Swirl to mix.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from red to pure blue.

Write down the number of digits displayed on the counter.

8. Use the multiplier in the *Range-specific information—mg/L* table (or the *Range-specific information—G.d.h.* table) to calculate the concentration:

digits x multiplier = mg/L (or G.d.h.) Ca as $CaCO_3$

Example:

50 mL of sample titrated with the 0.800 M EDTA titration cartridge, and 250 digits to reach the endpoint yields a calcium concentration of 250 x 2.0 = 500 mg/L as $CaCO_3$ (or with the 0.714 M EDTA titration cartridge, 250 x 0.1 = 25 mg/L G.d.h.)

Table 157 Range-specific information—mg/L

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Titration cartridge (M EDTA)	Multiplier
10–40	100	0.0800	0.1
40–160	25	0.0800	0.4
100–400	100	0.800	1.0
200–800	50	0.800	2.0
500–2000	20	0.800	5.0
1000–4000	10	0.800	10.0

Table 158 Range-specific information—G.d.h.

Range (G.d.h as $CaCO_3$)	Sample volume (mL)	Titration cartridge (M EDTA)	Multiplier
1–4	100	0.1428	0.01
4–16	25	0.1428	0.04
10–40	50	0.714	0.1
25–100	20	0.714	0.25
> 100	10	0.714	0.5

Interferences

WARNING
Chemical hazard. Potassium cyanide is toxic. Always add it after the potassium hydroxide. Follow local hazardous waste regulations for disposal of all cyanide-containing waste.

An interfering substance can prevent the color change at the titration end point. A dilution can often reduce the interference to a level at which the substance does not interfere. If an interference is suspected, decrease the sample volume, dilute to 100 mL and repeat the test.
Interfering substances lists substances that can interfere with this test.

Table 159 Interfering substances

Interfering substance	Interference level
Acidity	The test can tolerate 10,000 mg/L acidity.
Alkalinity	The test can tolerate 10,000 mg/L alkalinity .
Aluminum	Interferes by causing a slow end point but up to 200 mg/L aluminum can be tolerated by allowing sufficient time for the color change.
Barium	Barium is titrated along with calcium but is seldom found in natural waters in significant amounts.
Chloride	Saturated solutions do not give a distinct end point. The test can be run directly in sea water
Cobalt	Interferes at all levels. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 20 mg/L cobalt.
Copper	Interferes at 0.1 mg/L copper. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 100 mg/L copper.
Iron	Interferes above 8 mg/L by causing an orange-red to green end point. Accurate results can still be obtained up to approximately 20 mg/L iron with this end point.
Magnesium	Interference from magnesium is prevented up to 200 mg/L by the formation of magnesium hydroxide at the high test pH but higher levels prevent a distinct end point.
Manganese	Interferes above 5 mg/L.

Table 159 Interfering substances (continued)

Interfering substance	Interference level
Nickel	Interferes at 0.5 mg/L nickel. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 200 mg/L nickel.
Orthophosphate	Causes a slow end point but does not interfere if the calcium phosphate that forms is allowed to redissolve during the titration.
Polyphosphates	Interfere directly and must be absent.
Strontium	Strontium is titrated along with calcium but is seldom found in natural waters in significant amounts.
Temperature	Samples at about 20 °C (68 °F) or colder should be titrated slowly near the end point to allow sufficient time for the color change.
Zinc	Interferes at 5 mg/L zinc. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 100 mg/L zinc.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents. Adjust the pH before starting the test (see *Sample collection, preservation and storage*).

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been washed with a detergent and rinsed with tap water.
- Rinse the bottles in 1:1 nitric acid solution and deionized water.
- The following storage instructions are necessary only when immediate analysis is not possible. To preserve the sample, add 1.5 mL of nitric acid per liter (or quart) of sample. Mix. Measure the sample pH to make sure that the pH is 2 or less. Add more nitric acid in 0.5-mL increments if necessary. Mix thoroughly and check the pH after each addition until the pH is 2 or less.
- Preserved samples can be stored at least six months at room temperature.
- Before running the test, adjust the sample to pH 7 by adding potassium hydroxide standard solution and mixing thoroughly.
- Correct the test result for volume additions.

Accuracy check

Use the standard additions method to find if the sample has an interference and to make sure that the user has followed the procedure correctly.

Standard additions method (sample spike)
Required for accuracy check:

- Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL and Pipet Tips

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Repeat steps 2 and 3.

5. Each 0.1 mL of standard that was added will use approximately 10 digits of the 0.800 M titration cartridge or 100 digits of the 0.0800 M titration cartridge to reach the endpoint (11 digits of 0.714 M or 56 digits of 0.1428 M titrant).

   If more or less titrant was used, the problem can be due to user technique, an interference (see *Interferences*) or a problem with reagents or apparatus.

Summary of method

The sample is made alkaline (pH 12 to 13) with potassium hydroxide to precipitate magnesium as magnesium hydroxide. CalVer 2 Calcium Indicator is added as an indicator and combines with any calcium to form a red color. As the EDTA is added, it will react with all the free calcium ions present. At the end point of the titration, when no free calcium ions are present, the EDTA will remove the calcium complexed with the indicator. The indicator will then change from red to blue.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
10–160 mg/L range—Reagent set (approximately 100 tests):			2447200
(1) CalVer 2 Calcium Indicator Powder Pillows	1 pillow	100/pkg	85299
(1) Potassium Hydroxide Standard Solution, 8 N	1–2 mL	100 mL MDB	28232H
(1) EDTA Titration Cartridge, 0.0800 M	varies	each	1436401
100–4000 mg/L range—Reagent set (approximately 100 tests):			2447500
(1) CalVer 2 Calcium Indicator Powder Pillows	1 pillow	100/pkg	85299
(1) Potassium Hydroxide Standard Solution, 8 N	1–2 mL	100 mL MDB	28232H
(1) EDTA Titration Cartridge, 0.800 M	varies	each	1439901
1–16 G.d.h. range—Reagent set (approximately 100 tests):			2447300
(1) CalVer 2 Calcium Indicator Powder Pillows	1 pillow	100/pkg	85299
(1) Potassium Hydroxide Standard Solution, 8 N	1–2 mL	100 mL MDB	28232H
(1) EDTA Titration Cartridge, 0.1428 M	varies	each	1496001
10–100+ G.d.h. range—Reagent set (approximately 100 tests):			2447400
(1) CalVer 2 Calcium Indicator Powder Pillows	1 pillow	100/pkg	85299
(1) Potassium Hydroxide Standard Solution, 8 N	1–2 mL	100 mL MDB	28232H
(1) EDTA Titration Cartridge, 0.714 M	varies	each	1495901

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842

Recommended standards

Description	Unit	Catalog number
Hardness Standard Solution, Voluette ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	218710

Optional reagents and apparatus

Description	Unit	Catalog number
CalVer® 2 Calcium Indicator Powder	113 g	28114H
Magnesium Standard Solution, 10-g/L as $CaCO_3$	29 mL	102233
Nitric Acid Solution, ACS	500 mL	15249
Nitric Acid Solution, 1:1	500 mL	254049
CDTA Magnesium Salt Powder Pillow	100/pkg	1408099
Magnesium Standard Solution, 10-g/L as $CaCO_3$	29 mL	102233
Hardness 2 Indicator Solution	100 mL	42532
HexaVer Hardness Titrant, 0.020 N	1 L	74053
ManVer 2 Hardness Indicator Powder	113 g	28014
Nitric Acid Solution, ACS	500 mL	15249
Nitric Acid Solution, 1:1	500 mL	254049
Sodium Hydroxide Standard Solution, 5 N	50 mL	245026
Potassium cyanide	125 g	76714
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Tensette Pipet tips	50/pkg	218596
Potassium hydroxide, 8 N	500 mL	28249
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet, volumetric, Class A, 20 mL	each	1451520
Pipet Filler, safety bulb	each	1465100
Bottles, sampling, poly, 500 mL	each	2087079
Bromphenol Green-Methyl Red indicator solution	100 mL MDB	2329232
Phenolphthalein Indicator solution, 5 g/L	100 mL MDB	16232
pH meter	each	—
Pipet tips	50/pkg	2185696
Delivery Tube, 180° Hook	5/pkg	1720500
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Spoon, measuring, 1 g	each	51000
Spoon, measuring, 0.5 g	each	90700
Spoon, measuring, 0.1 g	each	51100
Voluette Ampule breaker 10 mL	each	2196800
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

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Edition 7

Hardness, Calcium

DOC316.53.01157

USEPA[1] Buret Titration Method[2]

Method 8222

0 to 25,000 mg/L as $CaCO_3$

Buret Titration

Scope and Application: For water, wastewater and seawater.

[1] USEPA accepted when 0.020 N titrant is used.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

A 0.1-g scoop of CalVer® 2 Calcium Indicator Powder can be used in place of the CalVer 2 Calcium Indicator Powder Pillow.

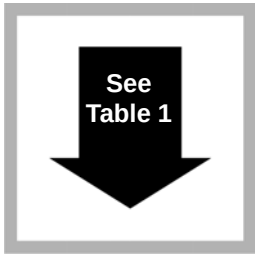
Magnesium is not included in the results but must be present for a sharp end point. If magnesium is not present, add one to two drops of Magnesium Standard Solution, 10-g/L as $CaCO_3$ to the sample before the test is started.

Collect the following items:

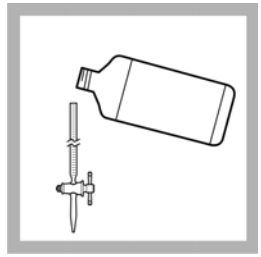
Description	Quantity
CalVer 2 Calcium Indicator Powder Pillow	1
Potassium Hydroxide Standard Solution, 8 N	1 mL
TitraVer Hardness Titrant (see *Range-specific information*)	1 bottle
Buret, Class A, 25-mL, with support stand	1
Erlenmeyer flask, 250 mL	1
Graduated cylinder	1

See *Consumables and replacement items* for reorder information.

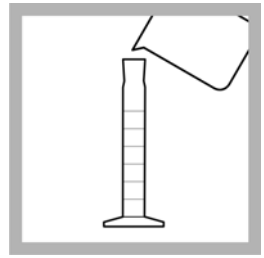
Buret titration



1. Select the sample volume and titrant concentration from the *Range-specific information* table.



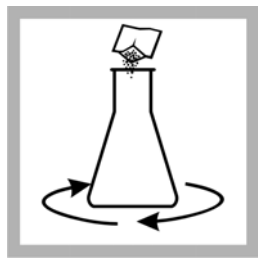
2. Fill a 25-mL buret to the zero mark with the TitraVer Hardness Titrant.



3. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table.

4. Transfer the sample into a 250-mL Erlenmeyer flask. If the sample volume is less than 50 mL, dilute to approximately 50 mL with deionized water. If magnesium is known to be absent, add one or two drops of Magnesium Standard Solution, 10-g/L as $CaCO_3$.

5. Add 1 mL of 8 N Potassium Hydroxide Standard Solution using the 1-mL dropper. Swirl to mix.



6. Add the contents of one CalVer 2 Calcium Indicator Powder Pillow. Swirl to mix.

7. Titrate the sample while swirling the flask until the color changes from red to pure blue.

Magnesium must be present (and usually is in natural waters) for a sharp end point, although it is not measured in the titration.

8. Use the multiplier in *Range-specific information* to calculate the concentration:

mL titrant used x multiplier = mg/L calcium as $CaCO_3$

Example: 50 mL of sample was titrated with the 0.020 N titrant solution and 15 mL of titrant was used to reach the endpoint.
The calcium concentration is: 15 x 20 = 300 mg/L as $CaCO_3$

Table 160 Range-specific information

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Hardness titrant concentration	Multiplier
0–500	50	0.020 N	20
400–1000	25	0.020 N	40
1000–2500	10	0.020 N	100
2000–5000	5	0.020 N	200
1000–5000	50	0.200 N	200
4000–10,000	25	0.200 N	400
10,000–25,000	10	0.200 N	1000

Table 161 Hardness conversions

To convert from	To	Multiply by
mg/L as $CaCO_3$	mg/L as Ca	0.400
	grains per gallon (gpg)	0.058
	German degrees hardness (Gdh)	0.056

Interferences

WARNING
Chemical hazard. Potassium cyanide is toxic. Always add it after the potassium hydroxide. Dispose of all cyanide containing wastes according to local regulations.

An interfering substance can prevent the color change at the titration end point. A dilution can reduce the interference to a level at which the substance does not interfere. If an interference is suspected, decrease the sample volume, dilute to 50 mL and repeat the test.
Interfering substances lists substances that can interfere with this test.

Table 162 Interfering substances

Interfering substance	Interference level
Acidity	The test can tolerate 10,000 mg/L acidity.
Alkalinity	The test can tolerate 10,000 mg/L alkalinity.
Aluminum	Interferes by causing a slow end point but up to 200 mg/L aluminum can be tolerated by allowing sufficient time for the color change.
Barium	Barium is titrated along with calcium but is seldom found in natural waters in significant amounts.
Chloride	Saturated solutions do not give a distinct end point, but the test can be run directly under sea water.
Cobalt	Interferes at all levels. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 20 mg/L cobalt.
Copper	Interferes at 0.1 mg/L copper. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 100 mg/L copper.
Iron	Interferes above 8 mg/L by causing an orange-red to green end point. Accurate results can still be obtained up to approximately 20 mg/L iron with this end point.
Magnesium	Interference from magnesium is prevented up to 200 mg/L by the formation of magnesium hydroxide at the high test pH but higher levels prevent a distinct end point.
Manganese	Interferes above 5 mg/L.
Nickel	Interferes at 0.5 mg/L nickel. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 200 mg/L nickel.

Table 162 Interfering substances (continued)

Interfering substance	Interference level
Orthophosphate	Causes a slow end point but does not interfere if the calcium phosphate that forms is allowed to redissolve during the titration.
Polyphosphates	Interfere directly and must be absent.
Strontium	Strontium is titrated along with calcium but is seldom found in natural waters in significant amounts.
Temperature	Samples at about 20 °C (68 °F) or colder should be titrated slowly near the end point to allow sufficient time for the color change.
Zinc	Interferes at 5 mg/L zinc. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 100 mg/L zinc.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents. Adjust the pH before starting the test (see *Sample collection, preservation and storage*).

Sample collection, preservation and storage

Collect samples in plastic or glass bottles that have been washed with a detergent and rinsed with tap water. Then rinse the bottles in 1:1 nitric acid solution and deionized water.

The following storage instructions are necessary only when immediate analysis is not possible. To preserve the sample, add 1.5 mL of nitric acid per liter (or quart) of sample. Mix. Measure the sample pH to make sure that the pH is 2 or less. Add more nitric acid in 0.5-mL increments if necessary. Mix thoroughly and check the pH after each addition until the pH is 2 or less. Preserved samples can be stored at least six months at room temperature.

Before running the test, adjust the sample to pH 7 by adding potassium hydroxide standard solution and mixing thoroughly. Correct the test result for volume additions.

Accuracy check

Use the standard additions method to find if the sample has an interference. Use the standard solution method to confirm anaytical technique and reagent performance.

Standard additions method (sample spike)
Required for accuracy check:

- Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL or 1.0–10.0 mL and Pipet Tips.

Procedure for use with the **0.020 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.

8. Each 0.1 mL of standard that was added should use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Accuracy check*).

Procedure for use with the **0.200 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 1.0 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 2.0 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 3.0 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 1.0 mL of standard that was added should use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Standard solution method
Complete the following test to confirm analytical technique and reagent performance.

Procedure for use with the **0.020 N** titrant:

1. Add 25.0 mL of a calcium chloride standard solution, 1000-mg/L as $CaCO_3$, to an Erlenmeyer flask. Dilute to 50 mL with deionized water and mix fully.
2. Add the potassium hydroxide and CalVer 2 indicator. Swirl to mix.
3. Titrate the standard to the end point with the 0.020 N hardness titrant and calculate the result. The titration should use 25 mL of titrant.

Procedure for use with the **0.200 N** titrant:

1. Add 10.0 mL of a Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$, to an Erlenmeyer flask. Dilute to 50 mL with deionized water and mix fully.
2. Add the potassium hydroxide and CalVer 2 indicator. Swirl to mix.
3. Titrate the standard to the end point with the 0.200 N hardness titrant and calculate the result. The titration should use 10 mL of titrant.

Summary of method

The sample is made alkaline (pH 12 to 13) with potassium hydroxide to precipitate magnesium as magnesium hydroxide. CalVer 2 Calcium Indicator is added as an indicator and combines with any calcium to form a red color. As the TitraVer (EDTA) is added, it will react with all the free calcium ions present. At the end point of the titration, when no free calcium ions are present, the EDTA will remove the calcium complexed with the indicator. The indicator will then change from red to blue.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hardness (Calcium) Reagent Set (approximately 100 tests), includes:			2447000
CalVer 2 Calcium Indicator Powder Pillows	1	100/pkg	85299
Potassium Hydroxide Standard Solution, 8 N	1 mL	100 mL MDB	28232H
TitraVer® Hardness Titrant, 0.020 N	varies	1 L	20553
TitraVer Hardness Titrant, 0.200 N	varies	500 mL	102149

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 5-mL	1	each	50837
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Support Stand	1	each	56300

Recommended standards and apparatus

Description	Unit	Catalog number
Calcium Chloride Standard Solution, 1000-mg/L as $CaCO_3$	1 L	12153
Hardness Standard Solution, Voluette ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	218710
Pipet, Class A volumetric, 10 mL	each	1451538
Pipet, Class A volumetric, 25 mL	each	1451540
Safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
CalVer® 2 Calcium Indicator Powder	113 g	28114H
Magnesium Standard Solution, 10-g/L as $CaCO_3$	29 mL	102233
Nitric Acid Solution, ACS	500 mL	15249
Nitric Acid Solution, 1:1	500 mL	254049
Potassium cyanide	125 g	76714
Potassium Hydroxide Standard Solution, 8 N	500 mL	28249
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Water, deionized	500 mL	27249
Spoon, measuring, 1 g	each	51000
Spoon, measuring, 0.5 g	each	90700
Spoon, measuring, 0.1 g	each	51100
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Voluette Ampule breaker 10 mL	each	2196800
Pipet, TenSette®, Pipet, 1.0–10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010[1]	50/pkg	2199796
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

[1] Other sizes are available

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Edition 7

Hardness, Total, Sequential

DOC316.53.01230

Titration Method using EDTA **Method 8329**

10 to 4000 mg/L as $CaCO_3$ **Digital Titrator**

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

The first titration gives calcium hardness and the second titration gives total hardness. The difference between the values is magnesium hardness. All of these concentrations are in mg/L as $CaCO_3$. See *Hardness conversions* for conversions to other units.
Four drops of Hardness 2 Indicator Solution or a 0.1-g scoop of ManVer 2 Hardness Indicator Powder can be added in place of the ManVer 2 Hardness Indicator Powder Pillow.
A 0.1-g scoop of CalVer® 2 Calcium Indicator Powder can be used in place of the CalVer 2 Calcium Indicator Powder Pillow.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

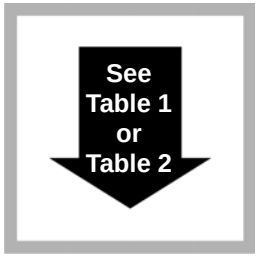
[1] See *Optional reagents and apparatus*.

Collect the following items:

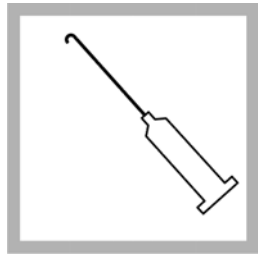
Description	Quantity
CalVer 2 Calcium Indicator Powder Pillow	1
Potassium Hydroxide Standard Solution, 8 N	1 mL
Hardness 1 Buffer Solution	1 mL
ManVer 2 Hardness Indicator Powder Pillow	1
Sulfuric Acid Standard Solution, 5.25 N	1 mL
EDTA titration cartridge (see *Range-specific information—mg/L* (mg/L) or *Range-specific information—G.d.h.* (German degrees of hardness))	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Erlenmeyer flask, 250 mL	1
Graduated cylinder	1

See *Consumables and replacement items* for reorder information.

Hardness, total, sequential



1. Select a sample volume and titration cartridge from *Range-specific information—mg/L* or *Range-specific information—G.d.h.*.



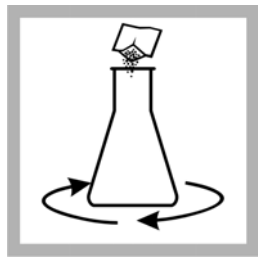
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

4. Use a graduated cylinder or pipet to measure the sample volume from *Range-specific information—mg/L* or *Range-specific information—G.d.h.*.

Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.

5. If the sample volume is 100 mL, add 2 mL of 8 N Potassium Hydroxide Standard Solution. If the sample volume is 50 mL or less, add 1 mL of 8 N Potassium Hydroxide Standard Solution. Swirl to mix.



6. Add the contents of one CalVer 2 Calcium Indicator Powder Pillow. Swirl to mix.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from red to pure blue.

Write down the number of digits on the counter.

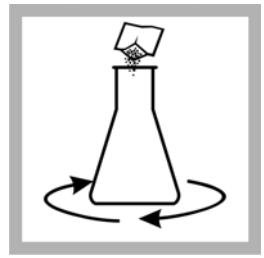
8. Use the multiplier in *Range-specific information—mg/L* (or *Range-specific information—G.d.h.*) to calculate the concentration:

Total digits from step 7 and step 12 x multiplier = mg/L (or G.d.h.) Calcium as $CaCO_3$

Hardness, total, sequential (continued)

9. Add 1 mL of 5.25 N Sulfuric Acid Standard Solution. Continue to add the acid by drops while swirling until the solution changes from pure blue to purple and finally to red. Swirl the flask to make sure that all the precipitated magnesium hydroxide has dissolved.

10. Add 2 mL of Hardness 1 Buffer Solution. Swirl to mix.



11. Add the contents of one ManVer 2 Hardness Indicator Powder Pillow. Swirl to mix.

12. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from red to pure blue.

Write down the number of digits on the counter.

13. Use the multiplier in *Range-specific information—mg/L* (or *Range-specific information—G.d.h.*) to calculate the concentration:

digits x multiplier = mg/L (or G.d.h.) total hardness as $CaCO_3$

Table 163 Range-specific information—mg/L

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Titration cartridge (M EDTA)	Multiplier
10–40	100	0.0800	0.1
40–160	25	0.0800	0.4
100–400	100	0.800	1.0
200–800	50	0.800	2.0
500–2000	20	0.800	5.0
1000–4000	10	0.800	10.0

Table 164 Range-specific information—G.d.h.

Range (G.d.h as $CaCO_3$)	Sample volume (mL)	Titration cartridge (M EDTA)	Multiplier
1–4	100	0.1428	0.01
4–16	25	0.1428	0.04
10–40	50	0.714	0.1
25–100	20	0.714	0.25
> 100	10	0.714	0.5

Table 165 Hardness conversions

To convert from	To	Multiply by
mg/L as $CaCO_3$	mg/L as Ca	0.400
	mg/L as Mg	0.243
	grains per gallon (gpg)	0.0584
	German degrees hardness (Gdh)	0.0559

Hardness relationships

mg/L Mg Hardness as $CaCO_3$

= mg/L Total Hardness as $CaCO_3$ – mg/L Ca Hardness as $CaCO_3$

mg/L $MgCO_3$ = mg/L Mg Hardness as $CaCO_3 \times 0.842$

mg/L Mg = mg/L $MgCO_3 \times 0.29$

Interferences

WARNING:
Do not use potassium cyanide to eliminate interferences because it will generate deadly hydrogen cyanide gas when the sulfuric acid solution is added.

- Although less common than calcium and magnesium, other polyvalent metal ions cause the same hardness effects and will be included in the results.
- Some transition and heavy metals complex the indicator and prevent the color change at the end point.

- Iron does not interfere up to 15 mg/L. Above this level it causes a red-orange to green end point which is sharp and usable up to 30 mg/L iron. Substitute a 0.0800 M CDTA or 0.800 M CDTA titration cartridge for the 0.0800 M EDTA or 0.800 M EDTA titration cartridges, respectively, if iron interference is probable. For results in G.d.h., divide the mg/L result by 17.9.
- Manganese titrates directly up to 20 mg/L but masks the end point above this level. Adding a 0.1-gram scoop of hydroxylamine hydrochloride raises this level to 200 mg/L manganese.
- Copper interferes at levels of 0.10 and 0.20 mg/L. Cobalt and nickel interfere at all levels and must be absent or masked.
- Orthophosphate causes a slow end point and polyphosphate must be absent for accurate results.
- Acidity and alkalinity at 10,000 mg/L (as $CaCO_3$) do not interfere.
- Saturated sodium chloride solutions do not give a distinct end point, but the titration can be run directly on sea water.
- Adding the contents of one CDTA Magnesium Salt Powder Pillow removes metal interferences at or below the levels shown in *Metal interferences*.
- If more than one metal is present at or above the concentrations shown in *Metal interferences*, an additional CDTA Magnesium Salt Powder Pillow may be required.
- Results obtained by this procedure include the hardness contributed by polyvalent metal ions. If the concentration of each metal is known, a correction can be applied to obtain the calcium and magnesium hardness concentration. The hardness (in mg/L as $CaCO_3$) contributed by each mg/L of metal is listed in *Hardness contributed by each mg/L of metal*. Hardness contributed by metals can be subtracted from the total hardness value obtained to determine the calcium and magnesium hardness concentration.
- Barium, strontium and zinc titrate directly.

Table 166 Metal interferences

Metal	CDTA Removes Interference Below this Level
Aluminum	50 mg/L
Cobalt	200 mg/L
Copper	100 mg/L
Iron	100 mg/L
Manganese	200 mg/L
Nickel	400 mg/L
Zinc	300 mg/L

Table 167 Hardness contributed by each mg/L of metal

Metal	Hardness as $CaCO_3$ Contributed by Each mg/L of Metal
Aluminum	3.710
Barium	0.729
Cobalt	1.698
Copper	1.575
Iron	1.792
Manganese	1.822
Nickel	1.705
Strontium	1.142
Zinc	1.531

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been washed with a detergent and rinsed with tap water. Then rinse the bottles in 1:1 nitric acid solution and deionized water.
- The following storage instructions are necessary only when immediate analysis is not possible. To preserve the sample, add 1.5 mL of nitric acid per liter (or quart) of sample. Mix.
- Measure the sample pH to make sure that the pH is 2 or less. Add more nitric acid in 0.5-mL increments if necessary.
- Mix thoroughly and check the pH after each addition until the pH is 2 or less.
- Store samples at 4 °C (39 °F) or below. Preserved samples can be stored for at least six months.
- Before starting the test, warm the sample to room temperature and adjust the pH to approximately pH 7 with potassium hydroxide solution. Mix thoroughly.
- If a significant amount of nitric acid was added, make a volume correction for the extra acid and hydroxide.
- Divide the total volume (sample + acid + hydroxide) by the volume of the sample and multiply the result from the test by this value.

Summary of method

This test procedure is a combination of the calcium and total hardness procedures. Refer to each method for a detailed description of the methods.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Calcium and Total Hardness Reagent Set (approximately 100 tests)			2272100
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
(1) CalVer 2 Calcium Indicator Powder Pillows	1	100/pkg	94799
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	85199
(2) Potassium Hydroxide Standard Solution, 8 N	1 mL	100 mL MDB	28232H
(1) EDTA Titration Cartridge, 0.0800 M	varies	each	1436401
(1) EDTA Titration Cartridge, 0.800 M	varies	each	1439901
Sulfuric Acid Standard Solution, 5.25 N	varies	100 mL MDB	244932

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Delivery tube for Digital Titrator, 180° hook	1	5/pkg	1720500
Delivery tube for Digital Titrator, 90° hook	1	5/pkg	4157800

Recommended standards

Description	Unit	Catalog number
Calcium Chloride Standard Solution, 1000-mg/L as $CaCO_3$	1 L	12153
Hardness Standard Solution, Voluette ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	218710
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
CalVer® 2 Calcium Indicator Powder	113 g	28114H
ManVer 2 Hardness Indicator Powder	113 g	28014
Nitric Acid Solution, ACS	500 mL	15249
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
pH paper, 0–14	100/pkg	2601300
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Weighing papers	500/pkg	1473800
Spoon, measuring, 0.1 g capacity	each	51100
Spoon, measuring, 0.5 g capacity	each	90700
Hydroxylamine Hydrochloride	113 g	24614
Magnesium CDTA powder pillows	100/pkg	1408099
CDTA cartridge for digital titrator, 0.08 M	each	1440201
CDTA cartridge for digital titrator, 0.80 M	each	1440301

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Edition 7

Hardness, Total

DOC316.53.01176

Titration Method using EDTA

Method 8213

10 to 4000 mg/L as $CaCO_3$

Digital Titrator

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

Four drops of Hardness 2 Indicator Solution or a 0.1-g scoop of ManVer 2 Hardness Indicator Powder can be added in place of the ManVer 2 Hardness Indicator Powder Pillow.
Hardness conversions: grains per gallon (gpg) as $CaCO_3$ = mg/L x 0.058; German degrees hardness (Gdh) = mg/L x 0.056; mg/L Total Hardness as Ca = mg/L Total Hardness as $CaCO_3$ x 0.40
mg/L Total Hardness as $CaCO_3$ = mg/L Ca as $CaCO_3$ + mg/L Mg as $CaCO_3$.
For added convenience when stirring, use the TitraStir® stirring apparatus[1].

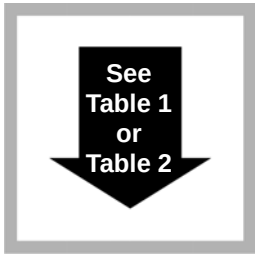
[1] See *Optional reagents and apparatus*.

Collect the following items:

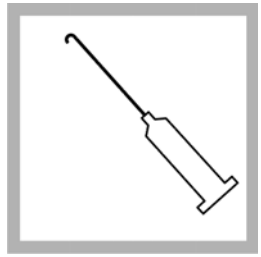
Description	Quantity
ManVer 2 Hardness Indicator Powder Pillow	1
Hardness 1 Buffer Solution	2 mL
EDTA titration cartridge (see *Range-specific information—mg/L* (mg/L) or *Range-specific information—G.d.h.* (German degrees of hardness))	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Hardness, Total



1. Select a sample volume and titration cartridge from the *Range-specific information—mg/L* table or the *Range-specific information—G.d.h.* table.



2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

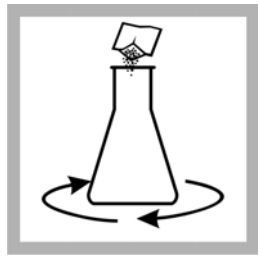
3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.

4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information—mg/L* table or the *Range-specific information—G.d.h.* table.

Transfer the sample into a clean, 250-mL Erlenmeyer flask. If the sample volume is less than 100 mL, dilute to approximately 100 mL with deionized water.

Hardness, Total

5. Add 2 mL of Hardness 1 Buffer Solution and swirl to mix.



6. Add the contents of one ManVer 2 Hardness Indicator Powder Pillow. Swirl to mix.

7. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from red to pure blue.

Write down the number of digits on the counter.

8. Use the multiplier in the *Range-specific information—mg/L* table (or the *Range-specific information—G.d.h.* table) to calculate the concentration:

digits x multiplier = mg/L (or G.d.h.) total hardness as $CaCO_3$

Example: 50 mL of sample was titrated with the 0.800 M EDTA titration cartridge and 250 digits were used to reach the endpoint. The total hardness concentration is: 250 x 2.0 = 500 mg/L as $CaCO_3$ (or with the 0.714 M EDTA titration cartridge, 250 x 0.1 = 25 mg/L G.d.h.)

Table 168 Range-specific information—mg/L

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Titration cartridge (M EDTA)	Multiplier
10–40	100	0.0800	0.1
40–160	25	0.0800	0.4
100–400	100	0.800	1.0
200–800	50	0.800	2.0
500–2000	20	0.800	5.0
1000–4000	10	0.800	10.0

Table 169 Range-specific information—G.d.h.

Range (G.d.h as $CaCO_3$)	Sample volume (mL)	Titration cartridge (M EDTA)	Multiplier
1–4	100	0.1428	0.01
4–16	25	0.1428	0.04
10–40	50	0.714	0.1
25–100	20	0.714	0.25
> 100	10	0.714	0.5

Interferences

WARNING
Chemical hazard. Potassium cyanide is toxic. Always add it after the potassium hydroxide. Follow local hazardous waste regulations for disposal of all cyanide-containing waste.

An interfering substance can prevent the color change at the titration end point. A dilution can often reduce the interference to a level at which the substance does not interfere. If an interference is suspected, decrease the sample volume, dilute to 100 mL and repeat the test. *Interfering substances* lists substances that can interfere with this test.

Table 170 Interfering substances

Interfering substance	Interference level
Acidity	The test can tolerate 10,000 mg/L acidity.
Alkalinity	The test can tolerate 10,000 mg/L alkalinity and can be run directly in sea water.
Aluminum	Aluminum interferes above 0.20 mg/L aluminum. 0.5 grams of potassium cyanide can be added after the buffer solution to remove interference from up to 1 mg/L aluminum.
Barium	Barium is included in the results but is seldom found in natural waters in significant amounts.
Cobalt	Interferes at all levels. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 20 mg/L cobalt.
Copper	Interferes at 0.1 mg/L copper. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 100 mg/L copper.
Iron	Interferes above 8 mg/L by causing an orange-red to green end point. Accurate results can still be obtained up to approximately 20 mg/L iron with this end point.
Manganese	Interferes above 5 mg/L.
Nickel	Interferes at 0.5 mg/L nickel. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 200 mg/L nickel.
Orthophosphate	Causes a slow end point but does not interfere if the calcium phosphate that forms is allowed to redissolve during the titration.
Polyphosphates	Interfere directly and must be absent.
Polyvalent metal ions	Although less common than calcium and magnesium, other polyvalent metal ions cause the same hardness effects and will be included in the results.
Sodium chloride	Saturated solutions do not give a distinct end point.
Strontium	Strontium is included in the results but is seldom found in natural waters in significant amounts.
Zinc	Interferes at 5 mg/L zinc. 0.5 grams of potassium cyanide can be added after the potassium hydroxide solution to remove interference from up to 100 mg/L zinc.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents. Adjust the pH before starting the test (see *Sample collection, preservation and storage*).

The addition of one CDTA Magnesium Salt Powder Pillow will remove metals interferences at or below the levels shown in *Interference level with CDTA pillow*. If more than one metal is present at or above the concentrations in *Interference level with CDTA pillow*, an additional CDTA Magnesium Salt Powder Pillow may be necessary.

Table 171 Interference level with CDTA pillow

Interfering substance	Interference level, mg/L
Aluminum	50
Cobalt	200
Copper	100
Iron	100
Manganese	200
Nickel	400
Zinc	300

The results obtained with CDTA Magnesium Salt will include the hardness contributed by the metals. If the concentration of each metal is known, a correction can be made to obtain the hardness contributed by calcium and magnesium only. The hardness contributed per mg/L metal ion is listed in *Hardness equivalence factors*. The mg/L of metal in the sample multiplied by its calcium carbonate hardness equivalent factor should be subtracted from the total hardness determined in step 3 of the procedure to obtain the hardness contributed by calcium and magnesium only.

Table 172 Hardness equivalence factors

Interfering substance	Hardness equivalence factors, mg/L as $CaCO_3$
Aluminum	3.710
Barium	0.729
Cobalt	1.698
Copper	1.575
Iron	1.792
Manganese	1.822
Nickel	1.705
Strontium	1.142
Zinc	1.531

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been washed with a detergent and rinsed with tap water.
- Rinse the bottles in 1:1 nitric acid solution and deionized water.
- The following storage instructions are necessary only when immediate analysis is not possible. To preserve the sample, add 1.5 mL of nitric acid per liter (or quart) of sample. Mix. Measure the sample pH to make sure that the pH is 2 or less. Add more nitric acid in 0.5-mL increments if necessary. Mix thoroughly and check the pH after each addition until the pH is 2 or less.
- Store samples at 4 °C (39 °F) or below. Preserved samples can be stored for at least seven days.
- Before starting the test, warm the sample to room temperature and adjust the pH to approximately pH 7 with 5.0 N sodium hydroxide.
- Mix thoroughly. If a significant amount of nitric acid was added, make a volume correction for the extra acid and hydroxide. Divide the total volume (sample + acid + hydroxide) by the volume of the sample and multiply the result from the test by this value.

Accuracy check

Use the standard additions method to determine whether the sample has an interference and to confirm the analytical technique.

Standard additions method (sample spike)
Required for accuracy check:

- Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL and Pipet Tips

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Repeat steps 2 and 3.
5. Each 0.1 mL of standard that was added will use approximately 10 digits of the 0.800 M titration cartridge or 100 digits of the 0.0800 M titration cartridge to reach the endpoint (11 digits of 0.714 M or 56 digits of 0.1428 M titrant).

   If more or less titrant was used, the problem can be due to user technique, an interference (see *Interferences*) or a problem with reagents or apparatus.

Standard solution method
Complete the following test to make sure that the reagents are user technique are accurate.

Required for accuracy check:

- Calcium Chloride Standard Solution, 1000-mg/L as $CaCO_3$

1. Add 20.0 mL of the standard solution to an Erlenmeyer flask. Dilute to about 100 mL with deionized water and mix fully.
2. Add the Hardness 1 Buffer Solution and ManVer 2 indicator. Swirl to mix.
3. Titrate the standard to the end point with the titration cartridge and calculate the result. The result should be close to 1000 mg/L or 55.9 G.d.h. as $CaCO_3$.

Summary of method

In the total hardness test, the water sample is first buffered (using an organic amine and one of its salts) to a pH of 10.1. An organic dye, calmagite, is added as the indicator for the test. This dye reacts with calcium and magnesium ions to give a red-colored complex.

EDTA (ethylenediaminetetraacetic acid) is added as a titrant. The EDTA will react with all free calcium and magnesium ions in the sample. At the end point of the titration, when free magnesium ions are no longer available, EDTA will remove magnesium ions from the indicator, causing a color change from red to blue.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
10–160 mg/L range—Reagent set (approximately 100 tests):			2448000
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	85199
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
(1) EDTA Titration Cartridge, 0.0800 M	varies	each	1436401
100–4000 mg/L range—Reagent set (approximately 100 tests):			2448100
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	85199
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
1–16 G.d.h. range—Reagent set (approximately 100 tests):			2447800
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	85199
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
(1) EDTA Titration Cartridge, 0.1428 M	varies	each	1496001
10–100+ G.d.h. range—Reagent set (approximately 100 tests):			2447900
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	85199
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
(1) EDTA Titration Cartridge, 0.714 M	varies	each	1495901
(1) EDTA Titration Cartridge, 0.800 M	varies	each	1439901

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838

Required apparatus (continued)

Description	Quantity/Test	Unit	Catalog number
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842

Recommended standards

Description	Unit	Catalog number
Calcium Chloride Standard Solution, 1000-mg/L as $CaCO_3$	1 L	12153
Hardness Standard Solution, Voluette ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	218710

Optional reagents and apparatus

Description	Unit	Catalog number
CDTA Magnesium Salt Powder Pillow	100/pkg	1408099
ManVer 2 Hardness Indicator Powder	113 g	28014
Magnesium Standard Solution, 10-g/L as $CaCO_3$	29 mL	102233
Hardness 2 Indicator Solution	100 mL	42532
HexaVer Hardness Titrant, 0.020 N	1 L	74053
Sodium Hydroxide Standard Solution, 5 N	50 mL	245026
Nitric Acid Solution, ACS	500 mL	15249
Nitric Acid Solution, 1:1	500 mL	254049
Potassium cyanide	125 g	76714
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Pipet tips	50/pkg	2185696
Pipet, Volumetric, 10 mL Class A	each	1451538
Pipet, Volumetric, 20 mL Class A	each	1451520
Pipet filler safety bulb	each	1465100
Delivery Tube, 180° Hook	5/pkg	1720500
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Spoon, measuring, 1 g	each	51000
Spoon, measuring, 0.5 g	each	90700
Spoon, measuring, 0.1 g	each	51100
Pipet Tips, for TenSette Pipet 1970010	50/pkg	2199796
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

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Edition 7

Hardness, Total

DOC316.53.01158

USEPA[1] ManVer 2 Buret Titration Method[2]

Method 8226

0 to 25,000 mg/L as $CaCO_3$

Buret Titration

Scope and Application: For water, wastewater and seawater.

[1] USEPA accepted when 0.020 N titrant is used.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

Four drops of Hardness 2 Indicator Solution or a 0.1-g scoop of ManVer 2 Hardness Indicator Powder can be added in place of the ManVer 2 Hardness Indicator Powder Pillow.

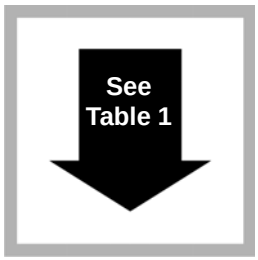
Hardness conversions: grains per gallon (gpg) as $CaCO_3$ = mg/L x 0.058; German degrees hardness (Gdh) = mg/L x 0.056

Collect the following items:

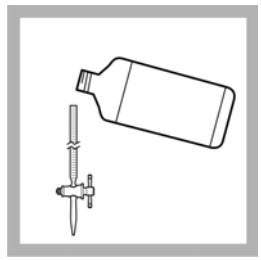
Description	Quantity
ManVer 2 Hardness Indicator Powder Pillow	1
Hardness 1 Buffer Solution	1 mL
TitraVer Hardness Titrant (see *Range-specific information*)	1 bottle
Buret, Class A, 25-mL, with support stand	1
Erlenmeyer flask, 250 mL	1
Graduated cylinder	1

See *Consumables and replacement items* for reorder information.

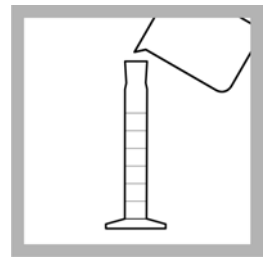
Buret titration



1. Select the sample volume and titrant concentration from *Range-specific information*.



2. Fill a 25-mL buret to the zero mark with the TitraVer Hardness Titrant.

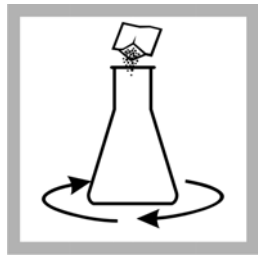


3. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table.

4. Transfer the sample into a 250-mL Erlenmeyer flask. If the sample volume is less than 50 mL, dilute to approximately 50 mL with deionized water.

Buret titration (continued)

5. Add 1 mL of Hardness 1 Buffer Solution using the 1-mL dropper. Swirl to mix.



6. Add the contents of one ManVer 2 Hardness Indicator Powder Pillow. Swirl to mix.

7. Titrate the sample while swirling the flask until the color changes from red to pure blue.

8. Use the multiplier in the *Range-specific information* to calculate the concentration:

mL titrant used x multiplier = mg/L total hardness as $CaCO_3$

Example: 50 mL of sample was titrated with the 0.020 N titrant and 15 mL of titrant was used to reach the endpoint. The hardness concentration is: 15 x 20 = 300 mg/L as $CaCO_3$

Table 173 Range-specific information

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Hardness titrant concentration	Multiplier
0–500	50	0.020 N	20
400–1000	25	0.020 N	40
1000–2500	10	0.020 N	100
1000–5000	50	0.200 N	200
4000–10,000	25	0.200 N	400
10,000–25,000	10	0.200 N	1000

Interferences

WARNING
Chemical hazard. Potassium cyanide is toxic. Always add it after the buffer solution. Dispose of all cyanide containing wastes according to local regulations

An interfering substance can prevent the color change at the titration end point. A dilution can reduce the interference to a level at which the substance does not interfere. If an interference is suspected, decrease the sample volume, dilute to 50 mL and repeat the test.

Interfering substances lists substances that can interfere with this test.

Table 174 Interfering substances

Interfering substance	Interference level
Acidity	Acidity at 10,000 mg/L as $CaCO_3$ does not interfere.
Alkalinity	Alkalinity at 10,000 mg/L as $CaCO_3$ does not interfere.
Aluminum	Aluminum interferes above 0.20 mg/L aluminum. 0.5 grams of potassium cyanide can be added after the buffer solution to remove interference from up to 1 mg/L aluminum.
Barium	Barium is titrated directly but is seldom found in natural waters in significant amounts.
Chloride	Saturated solutions do not give a distinct end point. The test can be run directly in sea water.
Cobalt	Interferes at all levels and must be absent or masked. 0.5 grams of potassium cyanide can be added after the buffer solution to remove interference from up to 100 mg/L cobalt.
Copper	Copper interferes above 0.10 mg/L copper. 0.5 grams of potassium cyanide can be added after the buffer solution to remove interference from up to 100 mg/L copper.
Iron	Interferes above 15 mg/L by causing an orange-red to green end point. Accurate results can still be obtained up to approximately 30 mg/L iron with this end point. For slightly sharper end points in solutions containing higher levels of iron, HexaVer Hardness Titrant (CDTA) can be used in place of the TitraVer Hardness Titrant (EDTA).
Manganese	Interferes above 20 mg/L. Adding a 0.10-gram scoop of Hydroxylamine Hydrochloride Monohydrate raises this level to 200 mg/L manganese.
Nickel	Interferes at all levels and must be absent or masked. 0.5 grams of potassium cyanide can be added after the buffer solution to remove interference from up to 100 mg/L nickel.
Orthophosphate	Causes a slow end point but does not interfere if the calcium phosphate that forms is allowed to redissolve during the titration.
Polyphosphates	Interfere directly and must be absent.
Polyvalent metal ions	Although less common than calcium and magnesium, other polyvalent metal ions cause the same hardness effects and will be included in the results.
Strontium	Strontium is titrated directly but is seldom found in natural waters in significant amounts.
Zinc	Titrates directly. 0.5 grams of potassium cyanide can be added after the buffer solution to remove interference from up to 100 mg/L zinc.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents. Adjust the pH before starting the test (see *Sample collection, preservation and storage*).

The addition of one CDTA Magnesium Salt Powder Pillow will remove metals interferences at or below the levels shown in the *Interference level with CDTA pillow* table. If more than one metal is present at or above the concentrations in the *Interference level with CDTA pillow* table, an additional CDTA Magnesium Salt Powder Pillow may be necessary.

Table 175 Interference level with CDTA pillow

Interfering substance	Interference level, mg/L
Aluminum	50
Cobalt	200
Copper	100
Iron	100
Manganese	200
Nickel	400
Zinc	300

The results obtained with CDTA Magnesium Salt will include the hardness contributed by those soluble metal ions present. If the concentration of soluble metal ion is known, a correction can be made to obtain the hardness contributed by calcium and magnesium only. The hardness

contributed per mg/L metal ion is listed in *Hardness equivalence factors* for the individual metals. The mg/L of metal present multiplied by its calcium carbonate hardness equivalent factor should be subtracted for each metal present from the total hardness determined in step 8 of the procedure to obtain the hardness contributed by calcium and magnesium only.

Table 176 Hardness equivalence factors

Interfering substance	Hardness equivalence factors, mg/L as $CaCO_3$
Aluminum	3.710
Barium	0.729
Cobalt	1.698
Copper	1.575
Iron	1.792
Manganese	1.822
Nickel	1.705
Strontium	1.142
Zinc	1.531

Sample collection, preservation and storage

Collect samples in plastic or glass bottles that have been washed with a detergent and rinsed with tap water. Then rinse the bottles in 1:1 nitric acid solution and deionized water.

The following storage instructions are necessary only when immediate analysis is not possible. To preserve the sample, add 1.5 mL of nitric acid per liter (or quart) of sample. Mix. Measure the sample pH to make sure that the pH is 2 or less. Add more nitric acid in 0.5-mL increments if necessary. Mix thoroughly and check the pH after each addition until the pH is 2 or less. Store samples at 4 °C (39 °F) or below. Preserved samples can be stored for at least seven days.

Before starting the test, warm the sample to room temperature and adjust the pH to approximately pH 7 with 5.0 N sodium hydroxide. Mix thoroughly. If a significant amount of nitric acid was added, make a volume correction for the extra acid and hydroxide. Divide the total volume (sample + acid + hydroxide) by the volume of the sample and multiply the result from the test by this value.

Accuracy check

Use the standard additions method to find if the sample has an interference. Use the standard solution method to confirm analytical technique and reagent performance.

Standard additions method (sample spike)
Required for accuracy check:

- Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL or 1.0–10.0 mL and Pipet Tips

Procedure for use with the **0.020 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.

5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 0.1 mL of standard that was added should use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Procedure for use with the **0.200 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 1.0 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 2.0 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 3.0 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 1.0 mL of standard that was added should use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Standard solution method

Complete the following test to confirm the analytical technique and reagent performance.

Procedure for use with the **0.020 N** titrant:

1. Add 25.0 mL of a calcium chloride standard solution, 1000-mg/L as $CaCO_3$, to an Erlenmeyer flask. Dilute to 50 mL with deionized water and mix fully.
2. Add the Hardness 1 Buffer Solution and ManVer 2 indicator. Swirl to mix.
3. Titrate the standard to the end point with the 0.020 N hardness titrant and calculate the result. The titration should use 25 (± 0.3) mL of titrant.

Procedure for use with the **0.200 N** titrant:

1. Add 10.0 mL of a Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$, to an Erlenmeyer flask. Dilute to 50 mL with deionized water and mix fully.
2. Add the Hardness 1 Buffer Solution and ManVer 2 indicator. Swirl to mix.
3. Titrate the standard to the end point with the 0.200 N hardness titrant and calculate the result. The titration should use 10 (± 0.2) mL of titrant.

Summary of method

In the total hardness test, the water sample is first buffered (using an organic amine and one of its salts) to a pH of 10.1. An organic dye, calmagite, is added as the indicator for the test. This dye reacts with calcium and magnesium ions to give a red-colored complex.

EDTA (ethylenediaminetetraacetic acid) is added as a titrant. The EDTA will react with all free calcium and magnesium ions present in the sample. At the end point of the titration, when free magnesium ions are no longer available, EDTA will remove magnesium ions from the indicator, causing a color change from red to blue.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hardness (Total) Reagent Set (approximately 100 tests), includes;			2447600
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	85199
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
(1) TitraVer® Hardness Titrant, 0.020 N	varies	1 L	20553
Hardness (Total) Reagent Set (approximately 100 tests), includes:			2447700
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	85199
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
(1) TitraVer® Hardness Titrant, 0.200 N	varies	500 mL	102149

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Support Stand	1	each	56300

Recommended standards and apparatus

Description	Unit	Catalog number
Calcium Chloride Standard Solution, 1000-mg/L as $CaCO_3$	1 L	12153
Hardness Standard Solution, Voluette ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	218710
Pipet, Class A volumetric, 10 mL	each	1451538
Pipet, Class A volumetric, 25 mL	each	1451540
Safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
CDTA Magnesium Salt Powder Pillow	100/pkg	1408099
Magnesium Standard Solution, 10-g/L as $CaCO_3$	29 mL	102233
Hardness 2 Indicator Solution	100 mL	42532
HexaVer Hardness Titrant, 0.020 N	1 L	74053
ManVer 2 Hardness Indicator Powder	113 g	28014
Nitric Acid Solution, ACS	500 mL	15249
Nitric Acid Solution, 1:1	500 mL	254049
Potassium cyanide	125 g	76714
Sodium Hydroxide Standard Solution, 5 N	50 mL	245026
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Water, deionized	500 mL	27249
Spoon, measuring, 1 g	each	51000
Spoon, measuring, 0.5 g	each	90700
Spoon, measuring, 0.1 g	each	51100
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Voluette Ampule breaker 10 mL	each	2196800
Pipet, TenSette®, Pipet, 1.0–10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010[1]	50/pkg	2199796
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

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Edition 7

Hardness, Total, Sequential

DOC316.53.01159

Buret Titration Method[1]

Method 8338

0 to 25,000 mg/L as $CaCO_3$

Buret Titration

Scope and Application: For water, wastewater and seawater.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

The first titration gives calcium hardness and the second titration gives total hardness. The difference between the values is magnesium hardness. All of these concentrations are in mg/L as $CaCO_3$. See *Hardness conversions* for conversions to other units.

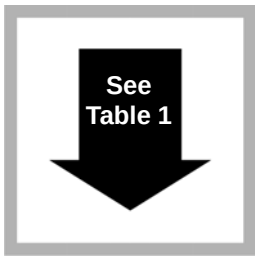
Two drops of Hardness 2 Indicator Solution can be added in place of the ManVer 2 Hardness Indicator Powder Pillow.

Collect the following items:

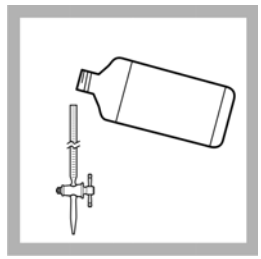
Description	Quantity
CalVer 2 Calcium Indicator Powder Pillow	1
Potassium Hydroxide Standard Solution, 8 N	1 mL
Hardness 1 Buffer Solution	1 mL
ManVer 2 Hardness Indicator Powder Pillow	1
Sulfuric Acid Standard Solution, 5.25 N	1 mL
TitraVer Hardness Titrant (see *Range-specific information*)	1 bottle
Buret, Class A, 25-mL, with support stand	1
Erlenmeyer flask, 250 mL	1
Graduated cylinder	1

See *Consumables and replacement items* for reorder information.

Buret titration



1. Select the sample volume and titrant concentration from *Range-specific information*.

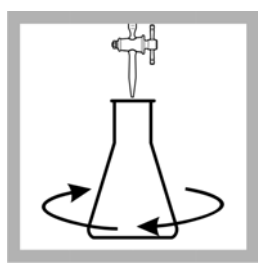


2. Fill a 25-mL buret to the zero mark with the TitraVer Hardness Titrant.

3. Use a graduated cylinder or pipet to measure the sample volume from *Range-specific information*. Transfer the sample into a 250-mL Erlenmeyer flask. If the sample volume is less than 50 mL, dilute to approximately 50 mL with deionized water.

4. Add 1 mL of 8 N Potassium Hydroxide Standard Solution using the 1-mL dropper. Swirl to mix.

5. Add the contents of one CalVer 2 Calcium Indicator Powder Pillow. Swirl to mix.

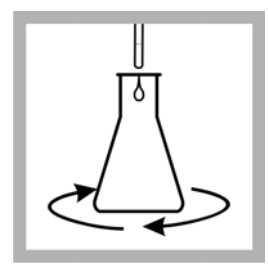


6. Titrate the sample while swirling the flask until the color changes from red to pure blue.

Magnesium must be present (and usually is in natural waters) for a sharp end point. Record the volume of titrant used.

7. Use the multiplier in *Range-specific information* to calculate the concentration:

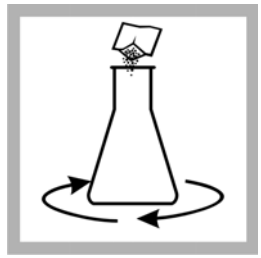
mL titrant used x multiplier = mg/L calcium as $CaCO_3$



8. Add 1 mL of 5.25 N Sulfuric Acid Standard Solution. Continue to add the acid by drops while swirling until the solution changes from pure blue to purple and finally to red. Swirl the flask to make sure that all the precipitated magnesium hydroxide has dissolved.

Buret titration (continued)

9. Add 1 mL of Hardness 1 Buffer Solution using the 1-mL dropper. Swirl to mix.



10. Add the contents of one ManVer 2 Hardness Indicator Powder Pillow. Swirl to mix.

11. Titrate the sample while swirling the flask until the color changes from red to pure blue.

Record the volume of titrant used.

12. Use the multiplier in *Range-specific information* to calculate the concentration:

Total mL titrant used in step 6 and step 11 x multiplier = mg/L total hardness as $CaCO_3$

Example: 50 mL of sample was titrated with the 0.020 N titrant and 15 mL of titrant was used to reach the endpoint. The hardness concentration is: 15 x 20 = 300 mg/L as $CaCO_3$

Table 177 Range-specific information

Range (mg/L as $CaCO_3$)	Sample volume (mL)	Hardness titrant concentration	Multiplier
0–500	50	0.020 N	20
400–1000	25	0.020 N	40
1000–2500	10	0.020 N	100
1000–5000	50	0.200 N	200
4000–10,000	25	0.200 N	400
10,000–25,000	10	0.200 N	1000

Table 178 Hardness conversions

To convert from	To	Multiply by
mg/L as $CaCO_3$	mg/L as Ca	0.400
	mg/L as Mg	0.243
	grains per gallon (gpg)	0.0584
	German degrees hardness (Gdh)	0.0559

Hardness relationships

mg/L Mg Hardness as $CaCO_3$

= mg/L Total Hardness as $CaCO_3$ – mg/L Ca Hardness as $CaCO_3$

mg/L $MgCO_3$ = mg/L Mg Hardness as $CaCO_3 \times 0.842$

mg/L Mg = mg/L $MgCO_3 \times 0.29$

Interferences

WARNING:
Do not use potassium cyanide to eliminate interferences because it will generate deadly hydrogen cyanide gas when the sulfuric acid solution is added.

- Although less common than calcium and magnesium, other polyvalent metal ions cause the same hardness effects and will be included in the results.
- Some transition and heavy metals complex the indicator and prevent the color change at the end point.
- Iron does not interfere up to 15 mg/L. Above this level it causes a red-orange to green end point which is sharp and usable up to 30 mg/L iron. For results in G.d.h., divide the mg/L result by 17.9.
- Manganese titrates directly up to 20 mg/L but masks the end point above this level. Adding a 0.1-gram scoop of hydroxylamine hydrochloride raises this level to 200 mg/L manganese.
- Copper interferes at levels of 0.10 and 0.20 mg/L. Cobalt and nickel interfere at all levels and must be absent or masked.
- Orthophosphate causes a slow end point and polyphosphate must be absent for accurate results.
- Acidity and alkalinity at 10,000 mg/L (as $CaCO_3$) do not interfere.
- Saturated sodium chloride solutions do not give a distinct end point, but the titration can be run directly on sea water.
- Adding the contents of one CDTA Magnesium Salt Powder Pillow removes metal interferences at or below the levels shown in the *Metal interferences* table.
- If more than one metal is present at or above the concentrations shown in the *Metal interferences* table, an additional CDTA Magnesium Salt Powder Pillow may be required.

- Results obtained by this procedure include the hardness contributed by polyvalent metal ions. If the concentration of each metal is known, a correction can be applied to obtain the calcium and magnesium hardness concentration. The hardness (in mg/L as $CaCO_3$) contributed by each mg/L of metal is listed in the *Hardness of each mg/L of metal* table. Hardness contributed by metals can be subtracted from the total hardness value to determine the calcium and magnesium hardness concentration.
- Barium, strontium and zinc titrate directly.

Table 179 Metal interferences

Metal	CDTA removes interference below this level
Aluminum	50 mg/L
Cobalt	200 mg/L
Copper	100 mg/L
Iron	100 mg/L
Manganese	200 mg/L
Nickel	400 mg/L
Zinc	300 mg/L

Table 180 Hardness of each mg/L of metal

Metal	Hardness as $CaCO_3$ contributed by each mg/L of Metal
Aluminum	3.710
Barium	0.729
Cobalt	1.698
Copper	1.575
Iron	1.792
Manganese	1.822
Nickel	1.705
Strontium	1.142
Zinc	1.531

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been washed with a detergent and rinsed with tap water. Then, rinse the bottles in 1:1 nitric acid solution and deionized water.
- The storage instructions are necessary only when immediate analysis is not possible. To preserve the sample, add 1.5 mL of nitric acid per liter (or quart) of sample. Mix.
- Measure the sample pH to make sure that the pH is 2 or less. Add more nitric acid in 0.5-mL increments if necessary.
- Mix thoroughly and check the pH after each addition until the pH is 2 or less.
- Store samples at 4 °C (39 °F) or below. Preserved samples can be stored for at least six months.
- Before starting the test, warm the sample to room temperature and adjust the pH to approximately pH 7 with potassium hydroxide solution. Mix thoroughly.
- If a significant amount of nitric acid was added, make a volume correction for the extra acid and hydroxide.

- Divide the total volume (sample + acid + hydroxide) by the volume of the sample and multiply the result from the test by this value.

Accuracy check

Use the standard additions method to find if the sample has an interference. Use the standard solution method to make sure that the user has followed the test correctly and that the reagents are good.

Standard additions method (sample spike)
Required for accuracy check:

- Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL

Procedure for use with the **0.020 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.1 mL of the standard to the titrated sample from step 7 or step 12. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.2 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 0.1 mL of standard that was added should use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Procedure for use with the **0.200 N** titrant:

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 1.0 mL of the standard to the titrated sample from step 7 or step 12. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 2.0 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 3.0 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 1.0 mL of standard that was added should use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Standard solution method
Complete the following test to make sure that the reagents are user technique are accurate.

Procedure for use with the **0.020 N** titrant:

1. Add 25.0 mL of a calcium chloride standard solution, 1000-mg/L as $CaCO_3$, to an Erlenmeyer flask. Dilute to 50 mL with deionized water and mix fully.
2. Add the reagents as shown in the test procedure. Swirl to mix.
3. Titrate the standard to the end point with the 0.020 N hardness titrant and calculate the result. The titration should use 25 (± 0.3) mL of titrant.

Procedure for use with the **0.200 N** titrant:

1. Add 10.0 mL of a Hardness Voluette Ampule Standard Solution, 10,000-mg/L as $CaCO_3$, to an Erlenmeyer flask. Dilute to 50 mL with deionized water and mix fully.
2. Add the reagents as shown in the test procedure. Swirl to mix.
3. Titrate the standard to the end point with the 0.200 N hardness titrant and calculate the result. The titration should use 10 (± 0.2) mL of titrant.

Summary of method

This test procedure is a combination of the calcium and total hardness procedures. Refer to each method for a detailed description of the methods.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hardness (Total) Reagent Set (approximately 100 tests), includes;			2448200
(1) Buffer Solution, Hardness 1	1 mL	100 mL MDB	42432
(1) CalVer 2 Calcium Indicator Powder Pillows	1	100/pkg	94799
(1) ManVer 2 Hardness Indicator Powder Pillows	1	100/pkg	92899
(1) Potassium Hydroxide Standard Solution, 8 N	1 mL	100 mL MDB	28232H
(1) Sulfuric Acid Standard Solution, 5.25 N	varies	100 mL MDB	244932
(1) TitraVer® Hardness Titrant, 0.020 N	varies	1 L	20553
TitraVer® Hardness Titrant, 0.200 N	varies	500 mL	102149

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Support Stand	1	each	56300

Recommended standards

Description	Unit	Catalog number
Calcium Chloride Standard Solution, 1000-mg/L as $CaCO_3$	1 L	12153
Hardness Standard Solution, Voluette ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	218710
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Nitric Acid Solution, ACS	500 mL	15249
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Water, deionized	500 mL	27249
pH paper, 0–14	100/pkg	2601300
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Spoon, measuring, 0.1 g capacity	each	51100
Spoon, measuring, 0.5 g capacity	each	90700
Hydroxylamine Hydrochloride	113 g	24614
Magnesium CDTA powder pillows	100/pkg	1408099
Hardness 2 indicator solution	100 mL MDB	42532

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Edition 7

Hydrazine

DOC316.53.01046

p-Dimethylaminobenzaldehyde Method[1] **Method 8141**

4 to 600 µg/L N_2H_4 **Reagent Solution or AccuVac® Ampuls**

Scope and Application: For boiler water/feedwater.

[1] Adapted from *ASTM Manual of Industrial Water*, D1385-78, 376 (1979).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 181 Instrument-specific information

Instrument	Reagent solution		AccuVac Ampuls
	Sample cell	Cell orientation	Adapter
DR 6000	2495402	Fill line faces right	—
DR 5000	2495402	Fill line faces user	—
DR 3900	2495402	Fill line faces user	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	LZV584 (C)

Before starting the test:

Samples cannot be preserved and must be analyzed immediately
Sample temperature should be 21 ± 4 °C (70 ± 7 °F).
After adding the HydraVer® 2 Hydrazine Reagent, a yellow color will develop in the sample if hydrazine is present. The blank may also have a faint yellow color.

Important Note: *The final samples will have a pH less than 2, which is considered corrosive (0002) by the Federal RCRA. Refer to the MSDS for disposal instructions.*

Collect the following items :

Description	Quantity
Solution Test:	
HydraVer 2 Reagent Solution	1 mL
Deionized Water	10 mL
Graduated Cylinder, 25 mL	1
AccuVac Test:	
HydraVer 2 Reagent AccuVac® Ampuls	2

Collect the following items (continued):

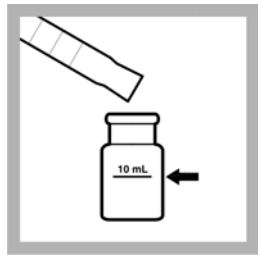
Description	Quantity
Deionized Water	40 mL
Beaker, 50 mL	1
Stopper for Ampules	2
See *Consumables and replacement items* for reorder information.	

Reagent solution method

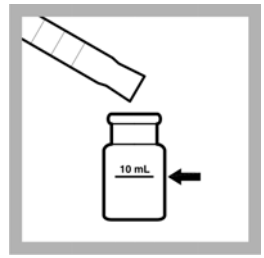
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

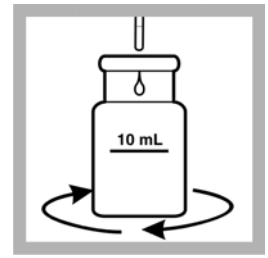
Refer to the user manual for orientation.



2. Blank Preparation: Use a graduated cylinder to pour 10 mL of deionized water into a sample cell.



3. Prepared Sample: Use a graduated cylinder to pour 10 mL of sample into a second sample cell.

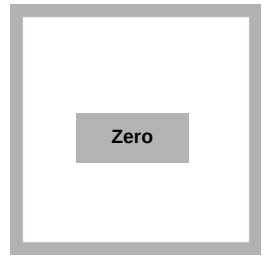


4. Add 0.5 mL of HydraVer 2 Hydrazine Reagent to each sample cell. Swirl to mix.

5. Start the instrument timer.

A 12-minute reaction period will begin. Complete steps 6–8 during this period.

6. Insert the blank cell.



7. **ZERO** the instrument.

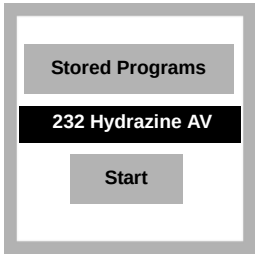
The display will show:

0 µg/L N_2H_4

8. Insert the prepared sample cell.

Immediately after the timer expires **READ** the results in µg/L N_2H_4.

AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

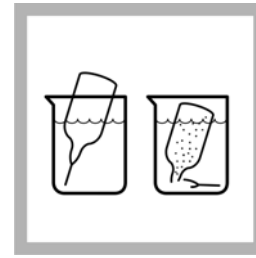
Refer to the user manual for orientation.

2. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill a HydraVer Hydrazine AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely. Cap with a stopper and mix.

3. Start the instrument timer.

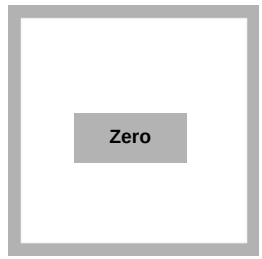
A 12-minute reaction period will begin. Complete steps 5–7 during this period.



4. Blank Preparation: Pour at least 40-mL of deionized water into a second beaker.

Fill a second Ampul with deionized water. Keep the tip immersed while the Ampul fills completely. Cap with a stopper and mix.

5. Insert the blank into the cell holder.



6. ZERO the instrument.

The display will show:

0 µg/L N_2H_4

7. Insert the prepared sample in the cell holder.

Immediately after the timer expires **READ** the results in µg/L N_2H_4.

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Interferences

Table 182 Interfering substances

Interfering substance	Interference level
Ammonia	No interference up to 10 mg/L. May cause a positive interference of up to 20% at 20 mg/L.
Highly colored or turbid samples	Prepare a blank by oxidizing the hydrazine in a portion of the sample with a 1:1 mixture of deionized water and household bleach. Add one drop of the mixture to 25 mL of sample in a graduated mixing cylinder and invert to mix. Use this solution in step 2, instead of deionized water, to prepare the blank.
Morpholine	No interference up to 10 mg/L.

Sample collection, preservation and storage

- Samples collected in glass or plastic bottles should be filled completely and capped tightly.
- Avoid excessive agitation or exposure to air.
- Samples must be analyzed immediately after collection and cannot be preserved for later analysis.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Prepare a 25 mg/L stock solution. Dissolve 0.1016 g of hydrazine sulfate in 1000 mL of oxygen-free deionized water. Prepare this stock solution daily.
2. Using Class A glassware, prepare a 0.25 mg/L (250 µg/L) hydrazine working solution by diluting 10.00 mL of the 25 mg/L stock solution to 1000 mL with deoxygenated deionized water. Prepare just before analysis. Perform either hydrazine procedure.
3. To adjust the calibration curve using the reading obtained with the 0.25-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
231	DR 5000	250 µg/L N_2H_4	247–253 µg/L N_2H_4	4 µg/L N_2H_4
232	DR 5000	250 µg/L N_2H_4	246–254 µg/L N_2H_4	4 µg/L N_2H_4

Summary of method

Hydrazine in the sample reacts with the p-dimethylaminobenzaldehyde from the HydraVer 2 Reagent to form a yellow color which is proportional to the hydrazine concentration. Test results are measured at 455 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
HydraVer® 2 Hydrazine Reagent	1 mL	100 mL MDB	179032
OR			
HydraVer 2 Hydrazine Reagent AccuVac® Ampuls	2	25/pkg	2524025
Water, deionized	10–40 mL	4 L	27256

Required apparatus (Reagent solution)

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 25-mL.	1	each	50840
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Required apparatus (AccuVac)

Description	Quantity	Unit	Catalog number
Beaker, 50 mL	1	each	50041H
Stopper	2	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Hydrazine Sulfate, ACS	100 g	74226

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, graduated mixing	each	189640
Flask, volumetric, Class A, 1000 mL	each	1457453
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet filler, Safety bulb	each	1465100
AccuVac Snapper	each	2405200

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Edition 7

Iodine

DOC316.53.01047

DPD Method [1] **Method 8031**

0.07 to 7.00 mg/L **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For testing dissolved iodine residual used as disinfectant in process water, treated water, estuary water and seawater

[1] Adapted from Palin, A.T., *Inst. Water Eng.*, 21 (6), 537-547 (1967).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 183 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

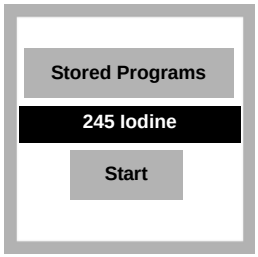
Analyze samples immediately. Do not preserve for later analysis
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Use DPD Total Chlorine reagents. Do not use DPD Free Chlorine reagents for this test.
If the sample temporarily turns yellow after reagent addition, dilute a fresh sample. Repeat the test. A slight loss of iodine may occur due to the dilution. Apply the appropriate dilution factor.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
DPD Total Chlorine Reagent Powder Pillow	1
Sample cells, 1-inch square, 10-mL (see *Instrument-specific information*)	2
AccuVac Test:	
DPD Total Chlorine Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1
Stopper for 18 mm Tube	2

See *Consumables and replacement items* for reorder information.

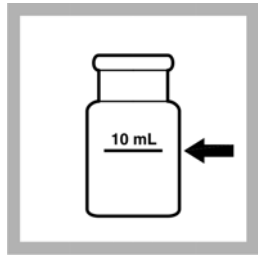
DPD for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. **Prepared sample:** Fill a sample cell with 10 mL of sample.

Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell.

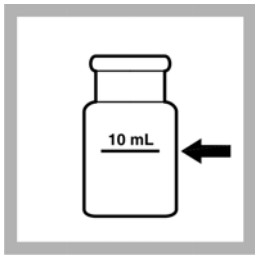
3. Swirl for about 20 second to mix.

A pink color will develop if iodine is present.

4. Start the instrument timer.

A three-minute reaction time will begin.

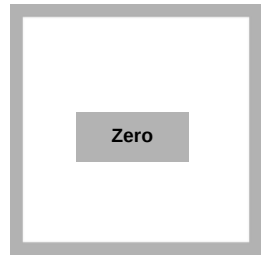
DPD for powder pillows (continued)



5. Blank preparation: Fill a second sample cell with 10 mL of sample.

6. Wipe the blank and insert it into the cell holder. (See *Instrument-specific information*.)

Close the cover.



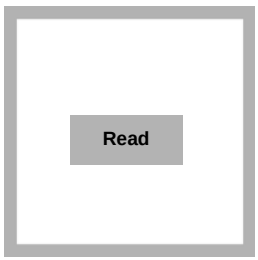
7. ZERO the instrument.

The display will show:

0.00 mg/L I_2

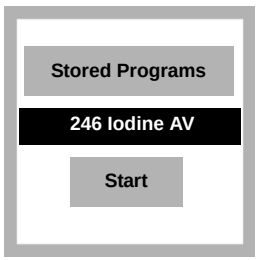
8. Within three minutes after the timer expires, wipe the prepared sample and insert it into the cell holder.

(See *Instrument-specific information*.)



9. READ the results in mg/L I_2.

DPD for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker. Fill a DPD Total Chlorine Reagent AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely.

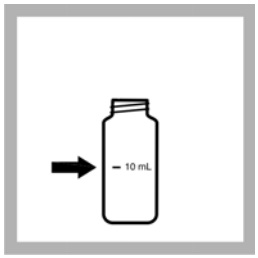
3. Quickly invert the Ampul several times to mix.

A pink color will develop if iodine is present.

4. Start the instrument timer.

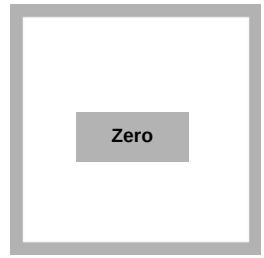
A three-minute reaction period will begin.

DPD for AccuVac® Ampuls (continued)



5. Blank Preparation: Fill a round sample cell with 10 mL of sample.

6. Wipe the blank and insert it into the cell holder.



7. **ZERO** the instrument.

The display will show:

0.00 mg/L I_2.

8. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L I_2.

Interferences

Table 184 Interfering substances

Interfering substance	Interference level
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 250 mg/L $CaCO_3$: May not develop full color or color may fade instantly. **1.** Neutralize to pH 6–7 with 1 N Sulfuric Acid[1]. **2.** Determine amount to be added on separate sample aliquot. Add the same amount to the sample to be tested. **3.** Correct for volume addition.
Bromine	Interferes at all levels
Chlorine and chloramines	Causes a positive interference at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as $CaCO_3$
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide[1] (30-g/L) to a 25-mL sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops Sodium Arsenite[1, 2] (5-g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct iodine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7.

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). Refer to the current MSDS for disposal information.

Sample collection, preservation and storage

- Collect samples in clean, dry glass containers.
- If sampling from a tap, allow the water to flow at least 5 minutes to ensure a representative sample.
- Avoid excessive agitation and exposure to sunlight when sampling.
- Allow several volumes of water to overflow the container and cap the container so there is no headspace above the sample.
- If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark.
- Proceed with the analysis immediately.

Accuracy check

Standard additions method (sample spike) for powder pillows
Required for accuracy check:

- LR Chlorine PourRite® Ampule Standard, 25–30 mg/L
- Ampule breaker
- TenSette Pipet and Pipet Tips

1. Analyze the sample and record the result.
2. Open the standard solution ampule.
3. Use the TenSette Pipet to prepare a spiked sample: add 0.1 mL of standard to a 10-mL portion of reacted sample. Swirl to mix.
4. Insert the spiked sample cell in the instrument.
5. Select Options>More>Standard Additions from the instrument menu.
6. Read the result.
7. Calculate the equivalent concentration of mg/L iodine added to the sample:

$$\text{mg/L Iodine} = \frac{0.1\ (\text{vol. standard added}) \times \text{mg/L Chlorine (certificate value)} \times 3.6}{10.1\ \ (\text{sample + standard volume})}$$

8. The spiked sample result from step 6 should reflect the analyzed sample result in step 1 plus the added, calculated mg/L iodine in step 7. If the result does not reflect the increase, refer to Standard Additions in the *Water Analysis Guide*.

Standard additions method (sample spike) for AccuVac Ampuls
Required for accuracy check:

- LR Chlorine PourRite® Ampule Standard, 25–30 mg/L
- DPD Total Chlorine AccuVac Ampul (2)
- Graduated cylinder
- Beakers
- Ampule breaker

- TenSette Pipet

1. Open the standard solution ampule.
2. Use a graduated cylinder to measure 25 mL of sample into a beaker. Use the TenSette Pipet to add 0.2 mL of standard to the beaker. Swirl to mix. This is the spiked sample.
3. Use a graduated cylinder to measure 25 mL of sample into a second beaker.
4. Fill an AccuVac Ampul from the spiked sample. Fill the second Ampul from the second beaker.
5. Follow the *DPD for AccuVac® Ampuls* test procedure.
6. Select Options>More>Standard Additions from the instrument menu.
7. Read the result.
8. Calculate the equivalent concentration of mg/L iodine added to the sample:

$$\text{mg/L Iodine} = \frac{0.2\ (\text{vol. standard added}) \times \text{mg/L Chlorine (certificate value)} \times 3.6}{25.2\ \ (\text{sample + standard volume})}$$

9. The spiked sample result should reflect the analyzed sample result plus the added, calculated mg/L iodine in step 8. If the result does not reflect the increase, refer to Standard Additions in the *Water Analysis Guide*.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
240	4.47 mg/L I_2	4.40–4.54 mg/L I_2	0.07 mg/L I_2
242	4.47 mg/L I_2	4.33–4.61 mg/L I_2	0.07 mg/L I_2

Summary of method

Iodine reacts with DPD (N, N-diethyl-p-phenylenediamine) to form a pink color, the intensity of which is proportional to the total iodine concentration. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
DPD Total Chlorine Reagent Powder Pillows	1	100/pkg	2105669
OR			
DPD Total Chlorine Reagent AccuVac® Ampuls	1	25/pkg	2503025

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional reagents and apparatus

Description	Unit	Catalog number
AccuVac Snapper Kit	each	2405200
Ampule Breaker Kit	each	2196800
Potassium Iodide, 30 g/L	100 mL MDB	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sodium Hydroxide, 1 N	100 mL MDB	104532
Stopper for 18 mm tube	25 / pkg	173125
Sulfuric Acid, 1 N	100 mL MDB	127032
Chlorine Standard, 25-30 mg/L ampules	20/pkg	2630020
Deionized Water	4 L	27256
TenSette Pipet, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette Pipet 1970001	50 / pkg	2185696
Pipet tips for TenSette Pipet 1970001	1000 / pkg	2185628

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Edition 7

Ferrozine® Method[1] **Method 8147**

0.009 to 1.400 mg/L **FerroZine Reagent Solution Pillows**

Scope and Application: For water and wastewater

[1] Adapted from Stookey, L.L., *Anal. Chem*., 42(7), 779 (1970)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 185 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

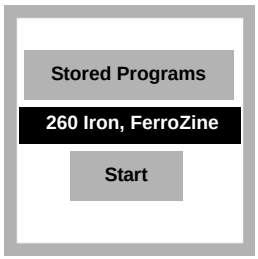
Digestion is required for total iron determination.
Rinse glassware with a 1:1 hydrochloric acid solution. Rinse again with deionized water. These two steps will remove iron deposits that can cause slightly high results.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Instead of solution pillows, 0.5 mL of FerroZine® Iron Reagent Solution can be used.
If the sample contains rust, see *Interferences*.
Use clean clippers, free of rust and wipe with a dry towel. Do not allow clippers to contact contents of the pillow.
FerroZine Iron Reagent may crystallize or precipitate when exposed to cold temperatures during shipment. Reagent quality is not affected. Place the reagent in warm water to redissolve.

Collect the following items:

Description	Quantity
FerroZine Iron Reagent Solution Pillows	1
OR	
FerroZine Iron Reagent Solution	0.5 mL
Cylinder, 25 mL graduated mixing, with stopper	1
Clippers for solution pillows	1
Sample Cells (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

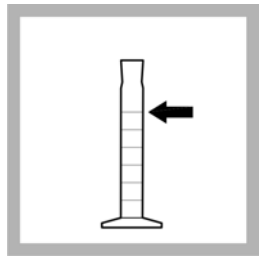
FerroZine solution pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



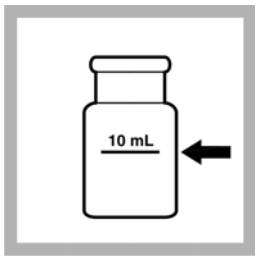
2. Fill a clean 25 mL graduated mixing cylinder to the 25 mL mark with sample.

3. Prepared sample: Add the contents of one FerroZine® Iron Reagent Solution Pillow to the mixing cylinder.

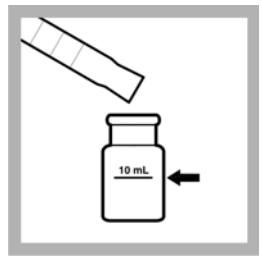
Stopper and invert to mix.

4. Start the instrument timer.

A five-minute reaction period will begin. A purple color will develop if iron is present.

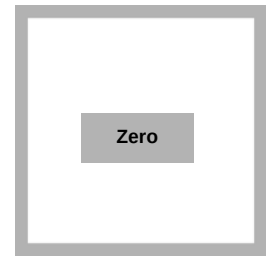


5. Blank preparation: Fill a sample cell with 10 mL of sample.



6. When the timer expires, pour 10 mL of the prepared sample into a second clean sample cell.

7. Insert the blank into the cell holder.



8. ZERO the instrument.

The display will show:

0.000 mg/L Fe

FerroZine solution pillows (continued)

9. Insert the prepared sample into the cell holder.

10. **READ** the results in mg/L Fe.

Interferences

Table 186 Interfering substances

Interfering substance	Interference level
Strong chelants (EDTA)	Interfere at all levels. Use the FerroVer® or TPTZ methods for these samples. Use the TPTZ method for low iron concentrations.
Cobalt	May give slightly high results
Copper	May give slightly high results
Hydroxides	Boil the sample, with the FerroZine® Iron Reagent added to it from step 3, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with step 4. Return the sample volume to 25 mL with deionized water.
Magnetite (black iron oxide) or Ferrites	**1.** Fill a 25 mL graduated cylinder with 25 mL of sample. **2.** Transfer this sample into a 125-mL Erlenmeyer flask. **3.** Add the contents of one FerroZine® Iron Reagent Solution Pillow and swirl to mix. **4.** Place the flask on a hot plate or over a flame and bring to a boil. **5.** Continue boiling gently for 20 to 30 minutes. ***Note:*** *Do not allow to boil dry. A purple color will develop if iron is present.* **6.** Return the boiled sample to the 25 mL graduated cylinder. Rinse the Erlenmeyer flask with small amounts of deionized water and empty into the graduated cylinder. **7.** Return the sample volume to the 25 mL mark with deionized water. **8.** Pour this solution into a sample cell and swirl to mix. **9.** Proceed with steps 5–10.
Rust	Boil the sample, with the FerroZine Iron Reagent added to it from step 3, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with step 4. Return the sample volume to 25 mL with deionized water.

Sample collection, preservation and storage

- Collect samples in acid-washed glass or plastic bottles.
- To preserve samples, adjust the sample pH to 2 or less with concentrated Nitric Acid, ACS* (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature.
- If only reporting dissolved iron, filter the sample immediately after collection and before adding nitric acid.
- Before testing, adjust the sample pH to 3–5 with Ammonium Hydroxide 10%.
- Do not exceed pH 5 or iron may precipitate.
- Correct test results for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Iron Voluette® Ampule Standard, 10 mg/L Fe
- Ampule breaker
- TenSette Pipet and Pipet Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Follow the *FerroZine solution pillows* test procedure for each of the spiked samples:
   a. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Start the instrument timer. After the timer expires, read the result.
   b. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Start the instrument timer. After the timer expires, read the result.
   c. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Start the instrument timer. After the timer expires, read the result. Each addition should reflect approximately 100% recovery.
6. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Iron Standard Solution, 100-mg/L
- 500 mL Class A volumetric flask
- Class A volumetric pipet, 5 mL and Pipet Filler

* See *Optional reagents and apparatus*.

- TenSette Pipet and Pipet Tips

1. Prepare a 1.0 mg/L Fe standard solution:
   a. Pipet 5.00 mL of Iron Standard, 100-mg/L into a 500-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
   c. Prepare this solution daily.
2. Follow the *FerroZine solution pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the 1.00 mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
260	DR 5000	1.000 mg/L Fe	0.985–1.015 mg/L Fe	0.009 mg/L Fe

Summary of method

The FerroZine® Iron Reagent forms a purple-colored complex with trace amounts of iron in samples that are buffered to a pH of 3.5. This method is applicable for determining trace levels of iron in chemical reagents and glycols and with digestion can be used to analyze samples containing magnetite (black iron oxide) or ferrites. Test results are measured at 562 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
FerroZine® Iron Reagent Solution	0.5 mL	500 mL	230149
OR			
FerroZine® Iron Reagent Solution Pillows	1	50/pkg	230166

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers for solution pillows	1	each	96800
Cylinder, graduated mixing, 25 mL with stopper	1	each	2088640
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards and apparatus

Description	Unit	Catalog number
Iron Standard Solution, 100 mg/L Fe	100 mL	1417542
Iron Standard Solution, 10 mL Voluette® ampule, 25 mg/L Fe	16/pkg	1425310
Metals Drinking Water Standard, LR for Cu, Fe, Mn	500 mL	2833749
Flask, volumetric, Class A, 500 mL	each	1457449
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, Class A, 5.00 mL	each	1451537
Pipet Filler, safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, 10%	100 mL MDB	1473632
Hydrochloric Acid, 1:1, 6N	500 mL	88449
Nitric Acid, ACS, concentrated	500 mL	15249

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Edition 7

Iron, Ferrous

DOC316.53.01049

1-10 Phenanthroline Method[1]

Method 8146

0.02 to 3.00 mg/L

Powder Pillows or AccuVac® Ampuls

Scope and Application: For water, wastewater and seawater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*, 15th ed. 201 (1980)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 187 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined.
If ferrous iron is present, an orange color will form after adding the reagent.

Collect the following items:

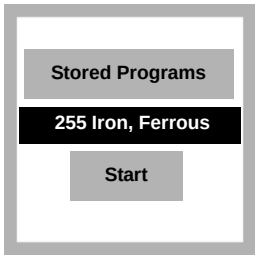
Description	Quantity
Powder Pillow Test:	
Ferrous Iron Reagent Powder Pillows	1
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
Ferrous Iron Reagent AccuVac® Ampuls	1
Beaker, 50 mL (AccuVac test)	1
Sample Cell (see *Instrument-specific information*)	1

Collect the following items:

Description	Quantity

See *Consumables and replacement items* for reorder information.

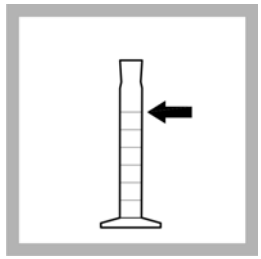
1-10 Phenanthroline method for powder pillows



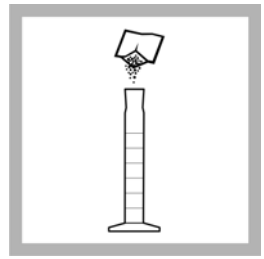
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

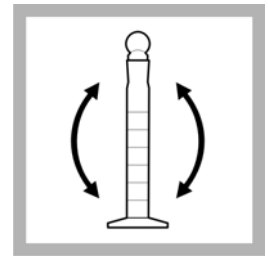
Refer to the user manual for orientation.



2. Fill a clean graduated mixing cylinder with 25 mL of sample.



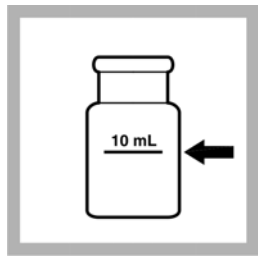
3. Prepared Sample: Add the contents of one Ferrous Iron Reagent powder pillow to the cylinder.



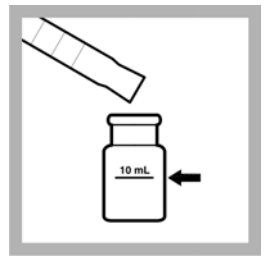
4. Insert a stopper and invert to mix. Undissolved powder does not affect accuracy.

5. Start the instrument timer.

A three-minute reaction period will begin.

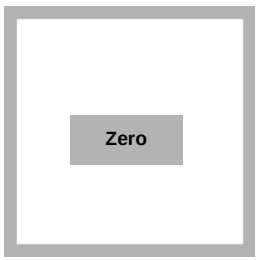


6. Blank Preparation: Fill a sample cell with 10 mL of sample.



7. Fill a second sample cell with the prepared sample from the mixing cylinder in step 4.

8. When the timer expires, insert the blank into the cell holder.

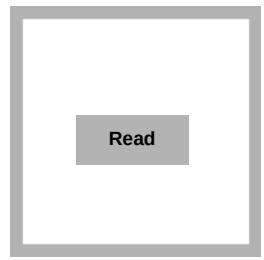


9. **ZERO** the instrument.

The display will show:

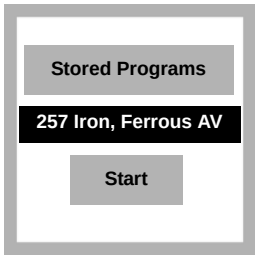
0.00 mg/L Fe^{2+}

10. Insert the prepared sample into the cell holder.



11. **READ** the results in mg/L Fe^{2+}.

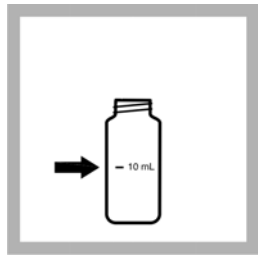
1-10 Phenanthroline method for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a round sample cell with 10 mL of sample.

3. Prepared Sample: Fill a Ferrous Iron Reagent AccuVac® Ampul with sample from the beaker. Keep the tip immersed while the Ampul fills completely.

4. Quickly invert the Ampul several times to mix.

5. Start the instrument timer.

A three-minute reaction period will begin.

6. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show:

0.00 mg/L Fe^{2+}

7. Wipe the Ampul and insert it into the cell holder.

READ the results in mg/L Fe^{2+}.

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles.
- Analyze samples as soon as possible after collection.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Ferrous Ammonium Sulfate, hexahydrate, 0.7022 g
- 1 L Class A volumetric flask
- 100 mL Class A volumetric flask
- Deionized water

- Analytical balance
- 2 mL Class A volumetric pipet and pipet filler

1. Prepare a 100 mg/L Fe^{2+} ferrous iron stock solution as follows:
   a. Dissolve 0.7022 grams of Ferrous Ammonium Sulfate, hexahydrate, in deionized water.
   b. Dilute to one liter in a Class A volumetric flask.
   c. In a 100 mL Class A volumetric flask, dilute 2.00 mL of this solution to 100 mL with deionized water to make a 2.0 mg/L standard solution. Prepare this solution immediately before use.
2. Follow the *1-10 Phenanthroline method for powder pillows* or the *1-10 Phenanthroline method for AccuVac® Ampuls* test procedure.
3. To adjust the calibration curve using the reading obtained with the 2.00-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
255	DR 5000	2.00 mg/L Fe^{2+}	1.99–2.01 mg/L Fe^{2+}	0.021 mg/L Fe^{2+}
257	DR 2800	2.00 mg/L Fe^{2+}	1.98–2.02 mg/L Fe^{2+}	0.023 mg/L Fe^{2+}

Summary of method

The 1-10 phenanthroline indicator in the Ferrous Iron Reagent reacts with ferrous iron (Fe^{2+}) in the sample to form an orange color in proportion to the iron concentration. Ferric iron (Fe^{3+})does not react. The ferric iron concentration can be determined by subtracting the ferrous iron concentration from the results of a total iron test. Test results are measured at 510 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ferrous Iron Reagent Powder Pillows	1	100/pkg	103769
OR			
Ferrous Iron Reagent AccuVac® Ampuls	1	25/pkg	2514025

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Beaker, 50 mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g x 0.1 mg 100–240 VAC	each	2936701
Ferrous Ammonium Sulfate, hexahydrate, ACS	113 g	1125614
Flask, volumetric, 1000 mL	each	1457453
Pipet filler, safety bulb	each	1465100
Pipet, volumetric, 2.00 mL	each	1451535
Water, deionized	4 L	27256
Wipers, disposable	280/pkg	2097000

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Edition 7

Iron, Total

DOC316.53.01053

USEPA[1] FerroVer® Method[2] **Method 8008**

0.02 to 3.00 mg/L **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater; digestion is required for determining total iron

[1] USEPA approved for reporting wastewater analysis, *Federal Register,* June 27, 1980; 45 (126:43459)

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 188 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

Digestion is required for determining total iron for EPA reporting purposes. Use the mild or vigorous digestion. Refer to the *Water Analysis Guide* for more information.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the user manual for more information.
Adjust pH of stored samples before analysis.
For turbid samples, treat the blank with one 0.1-g scoop of RoVer Rust Remover. Swirl to mix.

Collect the following items:

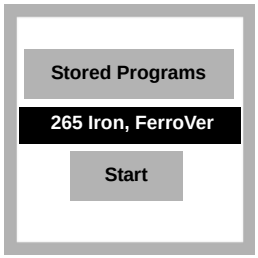
Description	Quantity
Powder Pillow Test:	
FerroVer® Iron Reagent Powder Pillow	1
Sample Cells (see *Instrument-specific information*)	2

Collect the following items: (continued)

Description	Quantity
AccuVac® Ampul test:	
FerroVer® Iron Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample Cells (see *Instrument-specific information*)	1
Stopper of 18 mm tubes	1

See *Consumables and replacement items* for reorder information.

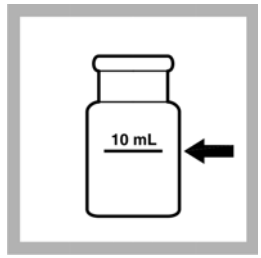
FerroVer method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Prepared sample: Fill a clean sample cell with 10 mL of sample

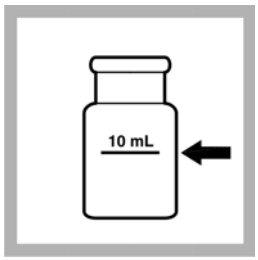
3. Add the contents of one FerroVer Iron Reagent Powder Pillow to the sample cell. Swirl to mix.

Accuracy is not affected by undissolved powder.

4. Start the instrument timer.

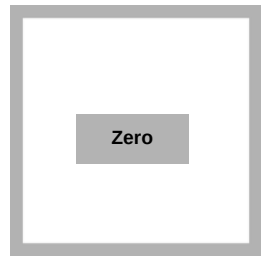
A three-minute reaction period will begin. An orange color will form, if iron is present.

(Allow samples that contain rust to react for at least 5 minutes.)



5. Blank preparation: Fill a second sample cell with 10 mL of sample.

6. When the timer expires, insert the blank into the cell holder.



7. ZERO the instrument.

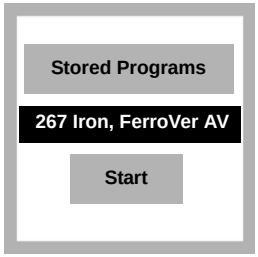
The display will show:

0.00 mg/L Fe

8. Insert the prepared sample into the cell holder.

READ the results in mg/L Fe.

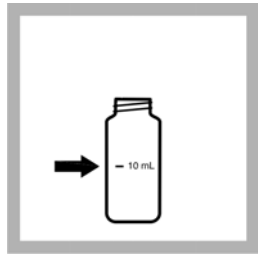
FerroVer method for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a round sample cell with 10 mL of sample.

3. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker. Fill a FerroVer Iron AccuVac® Ampul with sample from the beaker. Keep the tip immersed while the Ampul fills completely.

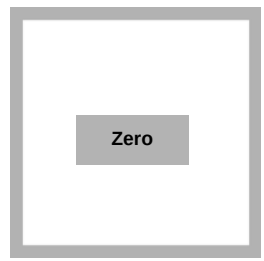
4. Quickly invert the Ampul several times to mix.

Accuracy is not affected by undissolved powder.

5. Start the instrument timer.

A three-minute reaction period will begin. An orange color will develop if iron is present.

6. Wipe the blank and insert it into the cell holder.



7. **ZERO** the instrument.

The display will show:

0.00 mg/L Fe

8. Wipe the Ampul and insert it into the cell holder.

READ the results in mg/L Fe.

Interferences

Table 189 Interfering substances

Interfering substance	Interference level
Calcium, Ca^{2+}	No effect at less than 10,000 mg/L as $CaCO_3$.
Chloride, Cl^-	No effect at less than 185,000 mg/L.
Copper, Cu^{2+}	No effect. Masking agent is contained in FerroVer Reagent.
High Iron Levels	Inhibit color development. Dilute sample and re-test to verify results.
Iron Oxide	Requires mild, vigorous or Digesdahl digestion. After digestion, adjust sample to pH 3–5 with sodium hydroxide, then analyze.
Magnesium	No effect at 100,000 mg/L as calcium carbonate.

Table 189 Interfering substances (continued)

Interfering substance	Interference level
Molybdate Molybdenum	No effect at 50 mg/L as Mo.
High Sulfide Levels, S^{2-}	**1.** Treat in fume hood or well-ventilated area. Add 5 mL hydrochloric acid[1], ACS to 100 mL sample in a 250 mL Erlenmeyer flask. Boil 20 minutes. **2.** Cool. Adjust pH to 3–5 with Sodium Hydroxide[1]. Readjust volume to 100 mL with deionized water. **3.** Analyze using *FerroVer method for powder pillows* or *FerroVer method for AccuVac® Ampuls*.
Turbidity	**1.** Add 0.1 g scoop of RoVer® Rust Remover to the blank. Swirl to mix. **2.** Zero the instrument with this blank. **3.** If sample remains turbid, add three 0.2 g scoops of RoVer to a 75 mL sample. Let stand 5 minutes. **4.** Filter through a Glass Membrane Filter and Filter Holder[1]. **5.** Use the filtered sample as the prepared sample and the blank.
Extreme Sample pH	Adjust pH to 3–5.
Highly Buffered Samples	Adjust pH to 3–5.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the sample immediately.
- To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.
- Before analysis, adjust the pH to between 3 and 5 with 5.0 N Sodium Hydroxide Standard Solution.
- Correct the test result for volume additions.
- If only dissolved iron is to be determined, filter the sample before acid addition.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Iron Voluette® Ampule Standard, 25 mg/L
- Ampule breaker
- TenSette Pipet and Pipet Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to 10 mL of unspiked sample. Start the instrument timer. After the timer expires, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Start the instrument timer. After the timer expires, read the result.

7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Start the instrument timer. After the timer expires, read the result.

8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)

1. Fill three mixing cylinders each with 50 mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of standard. Stopper and invert to mix.

2. Transfer 40 mL from each of the three mixing cylinders to three 50 mL beakers.

3. Analyze each standard addition sample as described in the *FerroVer method for AccuVac® Ampuls*.

4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Iron Standard Solution, 100 mg/L
- 100-mL volumetric flask
- Class A volumetric pipet, 2 mL
- Deionized water
- Pipet filler

1. Prepare a 2.00-mg/L Fe standard solution as follows:

   a. Pipet 2.00 mL of Iron Standard Solution, 100 mg/L, into a 100 mL volumetric flask.

   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.

2. Use the 2.00 mg/L Fe standard solution in place of the sample. Follow the *FerroVer method for powder pillows* test procedure.

3. To adjust the calibration curve using the reading obtained with the Standard Solution, select Options>More>Standard Adjust from the instrument menu.

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value. Mixed-parameter standards are also available to simulate various matrices.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
265	2.00 mg/L Fe	1.99–2.01 mg/L Fe	0.021 mg/L Fe
267	2.00 mg/L Fe	1.98–2.02 mg/L Fe	0.023 mg/L Fe

Summary of method

FerroVer Iron Reagent converts all soluble iron and most insoluble forms of iron in the sample to soluble ferrous iron. The ferrous iron reacts with the 1-10 phenanthroline indicator in the reagent to form an orange color in proportion to the iron concentration. Test results are measured at 510 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
FerroVer® Iron Reagent Powder Pillows (for 10-mL sample)	1	100/pkg	2105769
OR			
FerroVer® Iron Reagent AccuVac® Ampuls	1	25/pkg	2507025

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50 mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stopper for 18 mm tube	1	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Iron Standard Solution, 100 mg/L	100 mL	1417542
Iron Standard Solution, 10 mL Voluette® Ampule, 25 mg/L as Fe	16/pkg	1425310
Metals Drinking Water Standard, LR for Cu, Fe, Mn	500 mL	2833749
Metals Drinking Water Standard, HR for Cu, Fe, Mn	500 mL	2833649
Water, deionized	4 L	27256
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Flask, volumetric, Class A, 100 mL	each	1457442
Pipet, volumetric, Class A, 2.00 mL	each	1451536
Pipet Filler, safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
Beaker, 50 mL	each	50041H
Cylinder, mixing, 50 mL	each	189641
Hydrochloric Acid, concentrated	500 mL	13449
Nitric Acid, concentrated	500 mL	15249

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Sodium Hydroxide Standard Solution, 5.0 N	100 mL	245032
Glass Membrane Filter, 47 mm	100/pkg	253000
Glass Membrane Filter Holder	each	234000
RoVer Rust Remover	454 g	30001
Spoon, measuring, 0.1 g	each	51100

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Edition 7

Iron, Total

DOC316.53.01051

TPTZ Method[1] **Method 8112**

0.012 to 1.800 mg/L **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater

[1] Adapted from G. Frederic Smith Chemical Co., *The Iron Reagents,* 3rd ed. (1980)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 190 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

Digestion is required for determining total iron.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Rinse all glassware with a 1:1 Hydrochloric Acid Solution[1]. Rinse again with deionized water. This process will remove iron deposits that can cause slightly high results.
After adding reagent, a blue color will develop if iron is present.
Adjust the pH of stored samples to 3–4. Do not exceed pH 5 or iron may precipitate.

[1] See *Optional reagents and apparatus*.

Collect the following items:

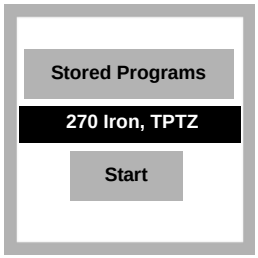
Description	Quantity
Powder Pillow Test:	
TPTZ Iron Reagent Powder Pillow	2
Sample Cells (see *Instrument-specific information*)	2

Collect the following items:

Description	Quantity
AccuVac Test:	
TPTZ Low Range Iron Reagent AccuVac®	1
Beaker, 50 mL	1
Stopper for 18 mm tube	1
Sample Cell (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

TPTZ method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

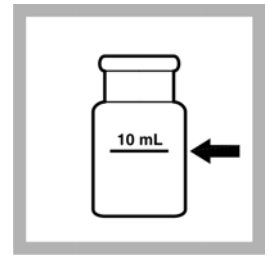
2. Prepared Sample: Fill one clean sample cell with 10 mL of sample.

Add the contents of one 10-mL TPTZ Iron Reagent Powder Pillow to the prepared sample. Swirl at least 30 seconds to dissolve.

3. Start the instrument timer.

A three-minute reaction period will begin.

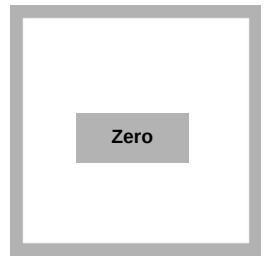
Proceed with step 4 and 5 while the timer is running.



4. Blank Preparation: Fill a second sample cell with 10 mL of deionized water.

5. Add the contents of one 10-mL TPTZ Iron Reagent Powder Pillow to the blank. Swirl at least 30 seconds to dissolve. This will be the reagent blank.

6. When the timer expires, insert the blank into the cell holder.



7. ZERO the instrument.

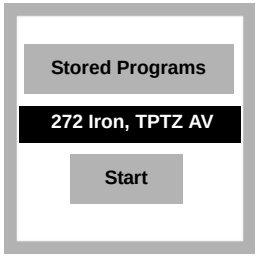
The display will show:

0.000 mg/L Fe

8. Insert the prepared sample into the cell holder.

READ the results in mg/L Fe.

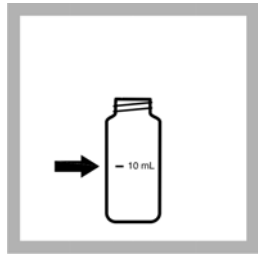
TPTZ method for AccuVac® Ampuls



1. Select the test.

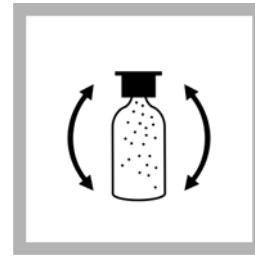
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a sample cell with 10 mL of sample.

3. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker. Fill a TPTZ Iron AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

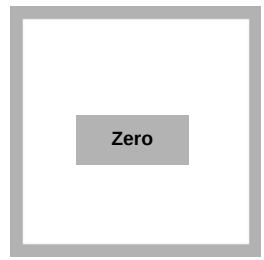


4. Quickly invert the Ampul several times to mix.

5. Start the instrument timer.

A three-minute reaction period will begin.

6. Wipe the blank and insert it into the cell holder.



7. **ZERO** the instrument.

The display will show:

0.000 mg/L Fe

8. Wipe the Ampul and insert it into the cell holder.

READ the results in mg/L Fe

Interferences

Interferences are tested (see the *Interfering substances* table) with an iron concentration of 0.5 mg/L. The following do not interfere with the method when present up to the levels given.

Table 191 Interfering substances

Interfering substance	Interference level
Cadmium	4.0 mg/L
Chromium $^{3+}$	0.25 mg/L
Chromium $^{6+}$	1.2 mg/L
Cobalt	0.05 mg/L
Copper	0.6 mg/L
Cyanide	2.8 mg/L
Manganese	50.0 mg/L
Mercury	0.4 mg/L
Molybdenum	4.0 mg/L
Nickel	1.0 mg/L
Nitrite Ion	0.8 mg/L
Color or turbidity	In the powder pillow procedure, if the sample, without a TPTZ Iron Reagent Powder Pillow, has a color or turbidity greater than the blank (deionized water plus TPTZ Iron Reagent), then use the sample as the blank.
pH	A sample pH of less than 3 or greater than 4 after the addition of reagent may inhibit color formation, cause the developed color to fade quickly or to result in turbidity. Adjust the sample pH in the sample cell before the addition of reagent: **1.** Measure the current pH by using a pH meter or pH paper. **2.** Adjust the sample pH to between 3 and 4 by adding an appropriate amount of iron-free acid or base such as 1.0 N Sulfuric Acid Standard Solution[1] or 1.0 N Sodium Hydroxide Standard Solution[1]. **3.** Make a volume correction if significant volumes of acid or base are used. Refer to the *Water Analysis Guide* for more information.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in acid-washed glass or plastic bottles.
- To preserve samples, adjust the sample pH to 2 or less with about 2 mL/L Nitric Acid, ACS*.
- Store preserved samples up to six months at room temperature.
- If reporting only dissolved iron, filter sample immediately after collection and before adding nitric acid.
- Before testing, adjust the pH of the stored sample to between 3–4 with 5.0 N Sodium Hydroxide Standard Solution*. Do not exceed pH 5 as iron may precipitate.
- Correct the test result for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method for powder pillows (sample spike)
Required for accuracy check:

- Iron Standard Solution, 10 mg/L Fe
- Beakers, 50 mL (3)
- Graduated mixing cylinders, 50 mL (3)
- TenSette Pipet

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a fresh bottle of standard solution.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
6. Follow the *TPTZ method for powder pillows* test procedure for each of the spiked samples using the powder pillows or AccuVac ampules, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)

1. Fill three mixing cylinders each with 50 mL of sample and spike with 0.5 mL, 1.0 mL and 1.5 mL of standard. Stopper and invert to mix.
2. Transfer 40 mL from each of the three mixing cylinders to three 50 mL beakers.
3. Analyze each standard addition sample as described in the *TPTZ method for AccuVac® Ampuls*.
4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Iron Standard Solution, 1 mg/L or
- Iron Standard Solution, 100 mg/L
- 5 mL Class A volumetric pipet and pipet filler
- 500 mL Class A volumetric flask
- Deionized water

1. Use a 1 mg/L Iron Standard Solution or prepare a 1.00 mg/L iron standard solution as follows:
   a. Pipet 5.00 mL of Iron Standard Solution, 100 mg/L, into a 500 mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.

2. Use the 1.00 mg/L iron standard solution in place of the sample. Follow the *TPTZ method for powder pillows* or Accuvac Ampuls.
3. To adjust the calibration curve using the reading obtained with the 1.00 mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value. Mixed-parameter standards are also available to simulate various matrices.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
270	1.000 mg/L Fe	0.989–1.011 mg/L Fe	0.011 mg/L Fe
272	1.000 mg/L Fe	0.984–1.016 mg/L Fe	0.012 mg/L Fe

Summary of method

The TPTZ Iron Reagent forms a deep blue-purple color with ferrous iron (Fe2+). The indicator is combined with a reducing agent that converts precipitated or suspended iron, such as rust, to the ferrous state. The amount of ferric iron (Fe3+) present can be determined as the difference between the results of a ferrous iron test and the concentration of total iron. Test results are measured at 590 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
TPTZ Iron Reagent Powder Pillows (for 10-mL sample)	1	100/pkg	2608799
OR			
TPTZ Low Range Iron Reagent AccuVac® Ampuls	1	25/pkg	2510025

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50 mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Iron Standard Solution, 100 mg/L Fe	100 mL	1417542
Iron Standard Solution, 10 mg/L Fe	500 mL	14049
Iron Standard Solution, 1 mg/L Fe	500 mL	13949
Metals Drinking Water Standard, LR for Cu, Fe, Mn	500 mL	2833749
Metals Drinking Water Standard, HR for Cu, Fe, Mn	500 mL	2833649
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 50 mL	each	189641
Nitric Acid, ACS concentrated	500 mL	15249
Sodium Hydroxide, 5.0 N	50 mL SCDB	245026
Sodium Hydroxide Standard Solution, 1.0 N	100 mL SCDB	127032
Sulfuric Acid, 1.0 N	100 mL MDB	104532
Stopper for 18 mm tube	6/pkg	173106
Tensette Pipet, 0.1–1.0 mL	each	1970001
Tips for Tensette Pipet	50/pkg	2185696
Flask, volumetric, 500 mL	each	1457449
Pipet, volumetric, 5 mL Class A	each	1451537
Pipet Filler, Safety bulb	each	1465100

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Edition 7

FerroZine® Rapid Liquid Method[1] **Method 8147**

0.009 to 1.400 mg/L Fe **Pour-Thru Cell**

Scope and Application: For boiler, cooling and natural waters

[1] Adapted from Stookey, L.L., *Anal.Chem.,* 42 (7) 779 1970.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 192 Instrument-specific information

Instrument	Pour-thru Kit	Cell orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

If sample contains rust, see *Interferences*.
Digestion is required for total iron determination.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
If iron is present, a purple color will form after adding the reagent
Rinse glassware with a 1:1 Hydrochloric acid[1] solution. Rinse again with deionized water. This will remove residual iron that may interfere
Prepare the Pour-Thru cell by preparing a solution of 1 mL Ferrozine Reagent per 50 mL of deionized water. Pour this into the cell and allow to stand for approximately 5 minutes to react with any trace iron in the cell and cell tubing. Flush with deionized water.
FerroZine Iron Reagent may crystallize or precipitate when exposed to cold temperatures during shipment; reagent quality is not affected. Place the reagent bottle in warm water to dissolve.
Make sure the Pour-Thru cell is completely seated in the sample cell compartment.

[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
FerroZine® Iron Reagent Solution	1.0 mL
Water, deionized	varies
Cylinder, graduated, 50 mL poly	1
Dispenser, fixed volume, 1.0 mL, with bottle	1
Flask, Erlenmeyer, PMP w/cap, 125 mL	2
Pour-Thru Cell Module (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

FerroZine Rapid Liquid Method for Pour-Thru Cell

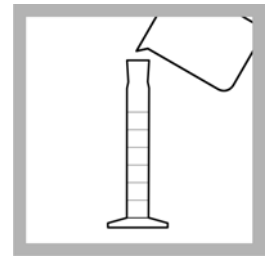
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

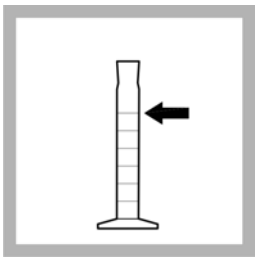
Refer to the user manual for orientation.

2. Flush the Pour-Thru Cell with 50 mL of deionized water.

3. Rinse two clean 125 mL Erlenmeyer flasks with the sample three times.



4. Rinse a clean 50-mL plastic graduated cylinder three times with the sample.



5. Fill the rinsed cylinder to the 50 mL mark with sample.

6. Pour the contents of the 50 mL cylinder into one of the flasks.

7. Prepared sample: Add 1.0 mL of FerroZine Iron Reagent Solution to flask using the Dispenser. Swirl to mix.

8. Start the instrument timer.

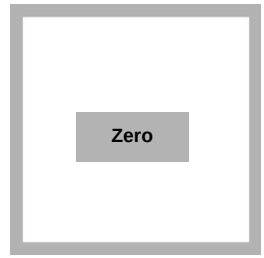
A five-minute reaction period will begin.

FerroZine Rapid Liquid Method for Pour-Thru Cell (continued)

9. Blank preparation: Measure a second 50 mL portion of sample into the graduated cylinder and pour the contents into the second flask.

10. When the timer expires the display will show “mg/L Fe.”

Pour the contents of the flask containing the blank into the Pour-Thru Cell



11. When the flow stops, **ZERO** the instrument.

The display will show:

0.000 mg/L Fe

12. Pour the contents of the flask containing the prepared sample into the Pour-Thru Cell.

13. When the flow stops, **READ** the results in mg/L Fe.

14. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Table 193 Interfering substances

Interfering substance	Interference level
Strong Chelants (EDTA)	Interfere at all levels. Use the FerroVer® or TPTZ methods for these samples. Use the TPTZ method for low iron concentrations.
Cobalt	May give slightly high results
Copper	May give slightly high results
Hydroxides	Boil the sample with the FerroZine® Iron Reagent added to it from step 7 of the test procedure for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with step 9. Return the sample volume to 50 mL with deionized water.
Magnetite (black iron oxide) or Ferrites	**1.** Fill a 50 mL graduated cylinder with 50 mL of sample. **2.** Transfer the sample into a clean glass 125 mL Erlenmeyer flask. **3.** Add 1.0 mL of FerroZine Iron Reagent Solution[1] and swirl to mix. **4.** Place the flask on a hot plate or over a flame and bring to a boil. **5.** Continue boiling gently for 20 to 30 minutes. ***Do not allow to boil dry.*** **6.** Return the boiled sample to the graduated cylinder. Rinse the Erlenmeyer flask with small amounts of deionized water and empty into the graduated cylinder. A purple color will develop if iron is present. **7.** Return the sample volume to the 50 mL mark with deionized water. **8.** Pour the solution into a 125 mL Erlenmeyer flask and swirl to mix. **9.** Proceed with steps 9–14 of the test procedure.
Rust	Boil the sample, with the FerroZine® Iron Reagent added to it from step 7, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with step 9. Return the sample volume to 50 mL with deionized water.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in acid-washed glass or plastic bottles.
- To preserve samples, adjust the sample pH to 2 or less with concentrated Nitric Acid, ACS* (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature.
- If only reporting dissolved iron, filter the sample immediately after collection and before adding nitric acid.
- Before testing, adjust the sample pH to 3–5 with Ammonium Hydroxide, 10%. Do not exceed pH 5 or iron may precipitate.
- Correct test results for volume additions. Refer to the *Water Analysis Guide* for more information.

* See *Optional reagents and apparatus*.

Labware

- All containers used in this test must be cleaned thoroughly to remove any traces of iron.
- Rinse labware and the Pour-Thru Cell with a 1:1 HCl solution* or with a 1:50 dilution of FerroZine® Reagent. Rinse several times with deionized water.
- Keep flasks tightly closed when not in use. Dedicate these containers for iron analysis only. If containers are rinsed and capped after each use, only occasional treatment with HCl or FerroZine® is necessary.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of color products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. Remove the color by rinsing with a 1:5 dilution of Ammonium Hydroxide*, followed by several rinses with deionized water. Cover the Pour-Thru Cell after use.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Iron Voluette® Ampule Standard, 25 mg/L Fe
- Ampule breaker
- TenSette Pipet and Pipet Tips
- Graduated mixing cylinder, 50 mL (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL and 0.6 mL of standard to three 50 mL portions of fresh sample.
6. Follow the *FerroZine Rapid Liquid Method for Pour-Thru Cell* test procedure for each of the spiked samples starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery. Each addition should reflect approximately 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 1.0 mg/L Iron Standard Solution or
- 100 mg/L Iron Standard Solution
- Deionized water
- 500 mL Class A volumetric flask

- 5 mL Class A volumetric pipet and filler

1. To check the accuracy, use a 1.0 mg/L Iron Standard Solution or prepare a 1.0 mg/L iron working solution as follows:
   a. Pipet 5.00 mL of iron standard solution, 100 mg/L Fe, into a 500 mL volumetric flask.
   b. Dilute to volume with deionized water. Prepare this solution daily.
2. Follow the *FerroZine Rapid Liquid Method for Pour-Thru Cell* test procedure.
3. To adjust the calibration curve using the reading obtained with the 1.00-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value. Mixed-parameter standards are also available to simulate various test matrices.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
261	1.000 mg/L Fe	0.997–1.003 mg/L Fe	0.009 mg/L Fe

Summary of method

The FerroZine® Iron Reagent forms a purple colored complex with trace amounts of iron in samples that are buffered to a pH of 3.5. This method is applicable for determining trace levels of iron in chemical reagents and glycols and with digestion can be used to analyze samples containing magnetite (black iron oxide) or ferrites. The test results are measured at 562 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
FerroZine® Iron Reagent Solution	1 mL	500 mL	230149
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 50-mL, poly	1	each	108141
Dispenser, fixed volume, 1.0-mL	1	each	2111302
Flask, Erlenmeyer, PMP w/cap, 125-mL	2	each	2089843

Recommended standards and apparatus

Description	Unit	Catalog number
Flask, volumetric, Class A, 500 mL	each	1457449
Iron Standard Solution, 100-mg/L Fe	100 mL	1417542
Iron Standard Solution, Voluette® ampule, 25-mg/L Fe, 10-mL	16/pkg	1425310
Iron Standard Solution, 1 mg/L Fe	500 mL	13949
Metals Drinking Water Standard, LR for Cu, Fe, Mn	500 mL	2833749
Pipet, TenSette 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, Class A, 5.00 mL	each	1451537
Pipet Filler, safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, ACS, 58%	500 mL	10649
Ammonium Hydroxide, ACS, 10%	100 mL MDB	1473632
Hydrochloric Acid, 1:1, 6N	500 mL	88449
Nitric Acid ACS, concentrated	500 mL	15249

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Edition 7

Iron, Total

DOC316.53.01052

FerroMo Method[1] **Method 8365**

0.01 to 1.80 mg/L **Powder Pillows**

Scope and Application: For cooling water containing molybdate-based treatment

[1] Adapted from G. Frederick Smith Chemical Co., *The Iron Reagents*, 3rd ed. (1980)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 194 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Rinse glassware with a 1:1 hydrochloric acid solution. Rinse again with deionized water. These two steps will remove iron deposits that can cause slightly high results.
After the addition of the reagent, the sample pH should be between 3–5.
If the sample contains high levels of molybdate (100 mg/L MoO_4^{2-} or greater), read the sample immediately after zeroing the blank.
Digestion is required for total iron determination.

Collect the following items:

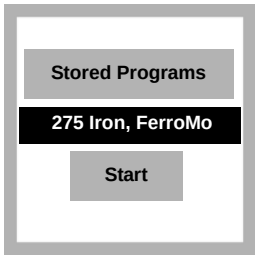
Description	Quantity
FerroMo® Reagent 1 Powder Pillow	1
FerroMo® Reagent 2 Powder Pillow	1
Cylinder, graduated mixing, 25 mL, with stopper	1
Cylinder, graduated mixing, 50 mL, with stopper	1
Sample Cells (see *Instrument-specific information*)	2

Collect the following items:

Description	Quantity

See *Consumables and replacement items* for reorder information.

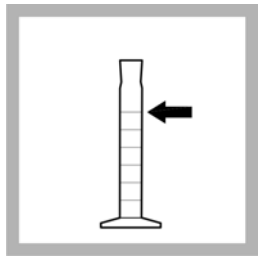
FerroMo method for powder pillows



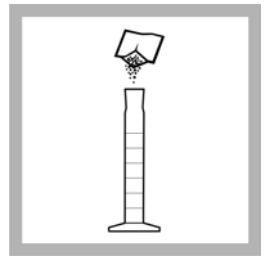
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

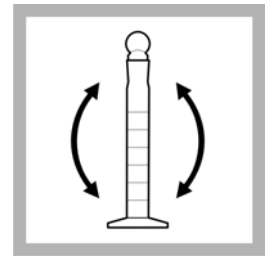
Refer to the user manual for orientation.



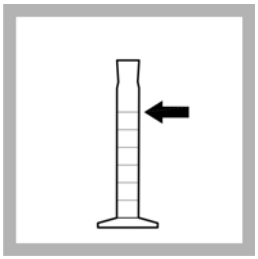
2. Prepared sample: Fill a 50-mL graduated mixing cylinder with 50 mL of sample.



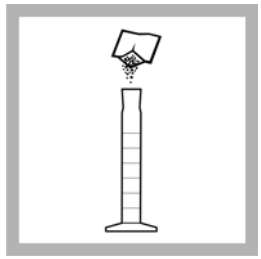
3. Add the contents of one FerroMo Iron Reagent 1 Powder Pillow to the graduated mixing cylinder. Insert the stopper.



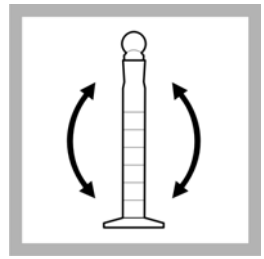
4. Invert several times to dissolve the reagents.



5. Fill a clean, 25-mL graduated cylinder to the 25-mL mark with prepared sample. Save the remaining prepared sample for step 10.



6. Developed sample: Add the contents of one FerroMo Iron Reagent 2 Powder Pillow to the sample in the 25-mL mixing cylinder.



7. Insert the stopper and invert to dissolve the reagents. A blue color will develop if iron is present.

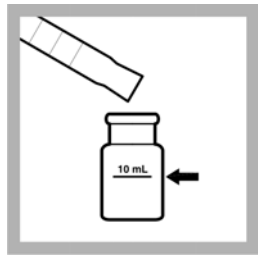
A small amount of undissolved reagent will not affect the results.

8. Start the instrument timer.

A three-minute reaction time will begin.

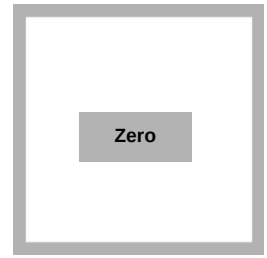
FerroMo method for powder pillows (continued)

9. When the timer expires, pour 10 mL of the developed sample from step 7 into a sample cell.



10. Blank preparation: Fill a second sample cell with 10 mL of the remaining prepared sample from step 5.

11. Insert the blank into the cell holder.

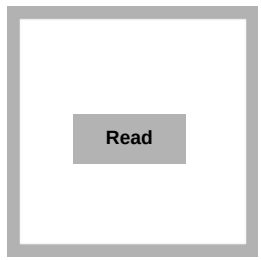


12. **ZERO** the instrument.

The display will show:

0.00 mg/L Fe

13. Insert the developed sample into the cell holder.



14. **READ** the results in mg/L Fe.

Interferences

Table 195 Interfering substances

Interfering substance	Interference level
pH	A sample pH of less than 3 or greater than 4 after the addition of reagent may inhibit color formation, cause the developed color to fade quickly or result in turbidity. Adjust the sample pH to between 3 and 8 in the graduated cylinder before the addition of reagent: **1.** Add by drops an appropriate amount of iron-free acid or base such as 1.0 N Sulfuric Acid Standard Solution[1] or 1.0 N Sodium Hydroxide Standard Solution[1]. **2.** Make a volume correction if significant volumes of acid or base are used. Refer to the *Water Analysis Guide* for more information.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in acid-washed glass or plastic bottles.
- To preserve samples, adjust the sample pH to 2 or less with hydrochloric acid (about 2 mL per liter)*. Samples preserved in this manner can be stored up to six months at room temperature.

* See *Optional reagents and apparatus*.

- If only dissolved iron is to be reported, filter sample immediately after collection through a 0.45 micron filter or equivalent medium before adding hydrochloric acid.
- Before testing, adjust the sample pH to 3–5 with 5.0 N Sodium Hydroxide Standard Solution*. Do not exceed pH 5 as iron may precipitate.
- Correct test results for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Iron Voluette® Ampule Standard, 50 mg/L Fe
- Mixing cylinders, 50 mL (3)
- Ampule breaker
- TenSette Pipet and Pipet Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 50 mL portions of fresh sample.
6. Follow the *FerroMo method for powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Iron Standard Solution, 1 mg/L or
- Iron Standard Solution, 100 mg/L
- 100 mL Class A volumetric flask
- 1 mL Class A volumetric pipet
- Deionized water

1. Use a 1.0 mg/L standard or prepare a 1.00 mg/L iron standard solution as follows:
   a. Pipet 1.0 mL of Iron Standard Solution, 100 mg/L, into a 100 mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the 1.00 mg/L iron standard solution in place of the sample. Follow the *FerroMo method for powder pillows* test procedure.

3. To adjust the calibration curve using the reading obtained with the 1.00-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
275	1.00 mg/L Fe	0.98–1.02 mg/L Fe	0.01 mg/L Fe

Summary of method

FerroMo Iron Reagent 1 contains a reducing agent combined with a masking agent. The masking agent eliminates interference from high levels of molybdate. The reducing agent converts precipitated or suspended iron, such as rust, to the ferrous state. FerroMo Iron Reagent 2 contains the indicator combined with a buffering agent. The indicator reacts with ferrous iron in the sample, buffered between pH 3 and 5, resulting in a deep blue-purple color. Test results are measured at 590 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
FerroMo® Iron Reagent Set (100 tests), includes:	—	—	2544800
(4) FerroMo® Reagent 1 Powder Pillows	1	25/pkg	2543768
(2) FerroMo® Reagent 2 Powder Pillows	1	50/pkg	2543866

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated mixing, 25 mL, with stopper	1	each	2088640
Cylinder, graduated mixing, 50 mL, with stopper	1	each	2088641
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Iron Standard Solution, 100 mg/L Fe	100 mL	1417542
Iron Standard Solution, 1 mg/L Fe	500 mL	13949
Iron Standard Solution, 10 mL Voluette® ampule, 50 mg/L Fe.	16/pkg	1425410
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Flask, volumetric, Class A, 100 mL	each	1457442
Pipet, volumetric, Class A, 1.00 mL	each	1451535
Pipet Filler, safety bulb	each	1465100
Sodium Hydroxide Standard Solution, 1.0 N	100 mL MDB	104532
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Sulfuric Acid Standard Solution, 1.0 N	100 mL MDB	127032
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Hydrochloric Acid, 1:1	500 mL	88449

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Edition 7

Iron

DOC316.53.01177

TitraVer Titration Method **Method 8214**

10 to 1000 mg/L as Fe **Digital Titrator**

Scope and Application: For water, wastewater and seawater.

Test preparation

Before starting the test:

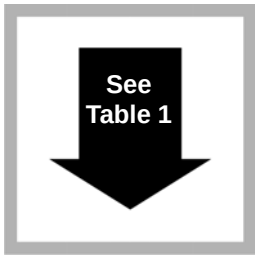
For added convenience when stirring, use the TitraStir® stirring apparatus.

Collect the following items:

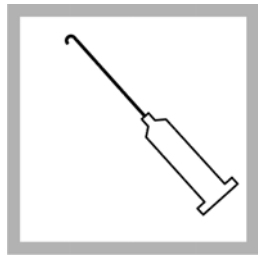
Description	Quantity
Citrate Buffer Powder Pillow	1 pillow
Sodium Periodate Powder Pillow	1 pillow
Sulfosalicylic Acid Powder Pillow	1 pillow
TitraVer Standard Solution titration cartridge (see *Range-specific information*)	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 125-mL	1

See *Consumables and replacement items* for reorder information.

Iron



1. Select a sample volume and titration cartridge from the *Range-specific information* table.



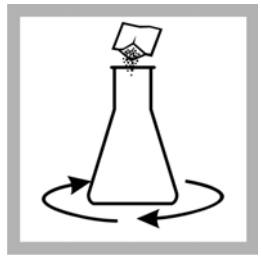
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

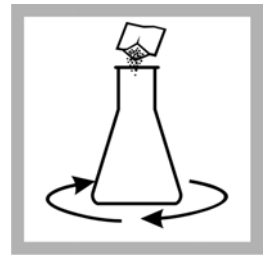
4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table in a 125 mL Erlenmeyer flask.

Iron

5. If the sample volume is less than 50 mL, dilute to approximately 50 mL with deionized water.

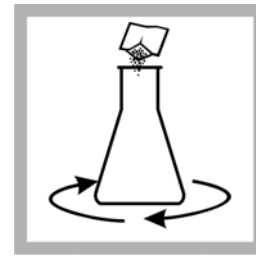


6. Add the contents of one Citrate Buffer Powder Pillow. Swirl to mix.



7. Add the contents of one Sodium Periodate Powder Pillow. Swirl to mix.

A yellow color will develop if iron is present.



8. Add the contents of one Sulfosalicylic Acid Powder Pillow. Swirl to mix.

A red color will develop if iron is present.

9. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from red to the original yellow.

Write down the number of digits displayed on the counter.

10. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L Fe

Example: 50 mL of sample was titrated with the 0.0716 N cartridge and 250 digits were used to reach the endpoint. The concentration is 250 x 0.1 = 25 mg/L Fe

Table 196 Range-specific information

Range (mg/L as Fe)	Sample volume (mL)	Titration cartridge (M TitraVer)	Multiplier
10–40	50	0.0716	0.1
25–100	20	0.0716	0.25
100–400	50	0.716	1.0
250–1000	20	0.716	2.5

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles.

Accuracy check

Use the standard additions method to determine whether the sample has an interference and to confirm the analytical technique.

Standard additions method (sample spike)
Required for accuracy check:

- Iron Standard Solution, 1000-mg/L as Fe
- TenSette Pipet, 0.1–1.0 mL

1. Use the TenSette Pipet to add 0.5 mL of the standard to the titrated sample. Swirl to mix.
2. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
3. Repeat steps 1 and 2.
4. Each 0.5 mL of standard that was added will use approximately 10 digits of the 0.716 M titration cartridge or 100 digits of the 0.0716 M titration cartridge to reach the endpoint.

   If more or less titrant was used, the problem can be due to user technique, an interference or a problem with reagents or apparatus.

Summary of method

Ferrous iron (Fe^{2+}) is oxidized by sodium periodate to ferric ion (Fe^{3+}). The ferric ion forms a red complex with sulfosalicylic acid. The red complex is destroyed by titration with EDTA. Citric acid is used to buffer the solution and to stabilize the ferric ion in solution.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
10–100 mg/L range—Reagent Set (approximately 100 tests):			2449200
(1) Citrate Buffer Powder Pillows	1 pillow	100/pkg	2081599
(1) Sodium Periodate Powder Pillows	1 pillow	100/pkg	98499
(1) Sulfosalicylic Acid Powder Pillows	1 pillow	100/pkg	2081669
(1) TitraVer Standard Solution Titration Cartridge, 0.0716 M	varies	each	2081701
100–1000 mg/L range—Reagent Set (approximately 100 tests):			2449300
(1) Citrate Buffer Powder Pillows	1 pillow	100/pkg	2081599
(1) Sodium Periodate Powder Pillows	1 pillow	100/pkg	98499
(1) Sulfosalicylic Acid Powder Pillows	1 pillow	100/pkg	2081669
(1) TitraVer Standard Solution Titration Cartridge, 0.716 M	varies	each	2081801

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Delivery tubes w/ 180° hook	1	each	1720500
Delivery tubes w/ 90° hook	1	each	4157800

Recommended standards

Description	Unit	Catalog number
Iron Standard Solution, 1000-mg/L as Fe	100 mL	227142

Optional reagents and apparatus

Description	Unit	Catalog number
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Bottle, sampling	250 mL	2087076
Iron standard, 10 mg/L	500 mL	14049
Iron standard, 25 mg/L	10 mL/16	1425310
Iron standard, 50 mg/L	10 mL/16	1425410
Iron standard, 100 mg/L	100 mL	1417542
Pipet tips	100/pkg	2185628
Pipet tips	50/pkg	2185696
Voluette breaker	each	2196800

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Edition 7

Lead

DOC316.53.01055

USEPA[1] Dithizone Method[2] **Method 8033**

3 to 300 µg/L **Powder Pillows**

Scope and Application: For water and wastewater

[1] USEPA accepted for reporting for wastewater analysis (digestion is required)

[2] Procedure is equivalent to Standard Method 3500-Pb D for wastewater analysis.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 197 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2612602	Fill line faces right
DR 5000	2612602	Fill line faces user
DR 3900	2612602	Fill line faces user
DR 3800, DR 2800, DR 2700	2612602	Fill line faces right

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.

Clean all glassware with a 1:1 Nitric Acid Solution. Rinse with deionized water.

Cloudy and turbid samples may require filtering before running the test. Report results as µg/L soluble lead. Use glass membrane type filter to avoid loss of lead by adsorption onto the filter paper.

If samples cannot be analyzed immediately, see *Sample collection, preservation and storage*. Adjust the pH of preserved samples before analysis.

For more accurate results, adjust the sample to pH 11.0–11.5 using a pH meter in step 11. Omit the five additional drops of Sodium Hydroxide Standard Solution in step 12

The DithiVer powder will not completely dissolve in the chloroform. For further notes see *DithiVer solution preparation, storage and reagent blank*.

Read the MSDS before testing. Spilled reagent will affect test accuracy and is hazardous to skin and other materials.

In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

Digestion is required to for determine the total lead for EPA reporting purposes. Use mild or vigorous digestion.

Collect the following items:

Description	Quantity
Citrate Buffer Powder Pillows	1
Chloroform	50 mL
DithiVer Metals Reagent Powder Pillows	1
Potassium Cyanide	2 g
Sodium Hydroxide Standard Solution, 5.0 N	varies
Cotton Balls	1
Clippers	1
Cylinder, 50 mL graduated mixing	1
Cylinder, 5 mL graduated	1
Cylinder, 50 mL graduated	1
Cylinder, 250 mL graduated	1
Funnel, 500 mL separatory	1
Sample Cells (see *Instrument-specific information*)	2
Spoon, measuring, 1.0 g	1
Support Ring (4 inch) and Stand (5 x 8 inch base)	1

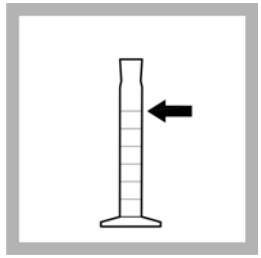
See *Consumables and replacement items* for reorder information.

Dithizone method for powder pillows

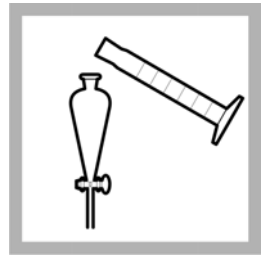
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



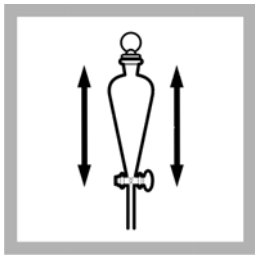
2. Fill a 250 mL graduated cylinder to the 250 mL mark with sample.



3. Transfer the sample into 500 mL separatory funnel.

4. Add the contents of one Buffer Powder Pillow, citrate type.

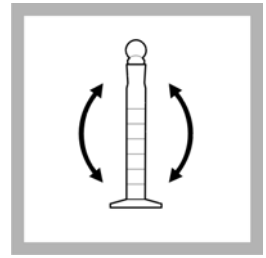
Dithizone method for powder pillows (continued)



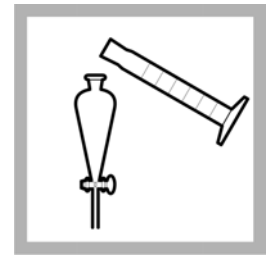
5. Insert the stopper into the funnel and shake to dissolve.

6. DithiVer Solution preparation:

Add 50 mL of chloroform to a 50-mL mixing graduated cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow.



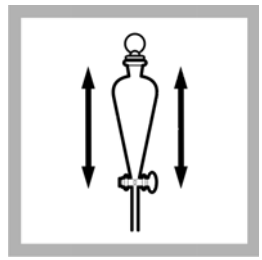
7. Stopper the cylinder. Invert several times to mix.



8. Measure 30 mL of the prepared dithizone solution with a second graduated cylinder and add to the separatory funnel.

Insert the stopper and invert to mix. Open stopcock to vent. Close the stopcock.

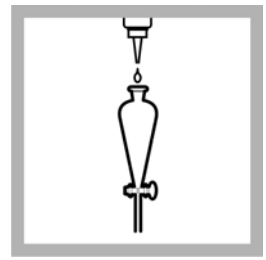
9. Add 5 mL of 5.0 N Sodium Hydroxide Standard Solution.



10. Stopper. Invert. Open stopcock to vent. Close the stopcock and shake the funnel once or twice and vent again.

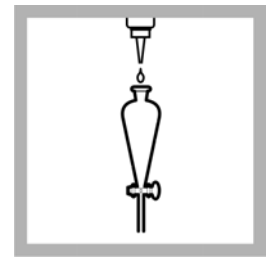
If the solution turns orange after shaking, the pH is too high. Add a few drops of 5.25 N Sulfuric Acid to the solution to decrease the pH.

The blue-green color will reappear (alternatively, to avoid higher blanks, repeat on new sample and use less sodium hydroxide in step 9).



11. Continue adding 5.0 N Sodium Hydroxide Standard Solution dropwise and shaking the funnel after every few drops until the color of the solution being shaken changes from blue-green to orange.

Large amounts of zinc cause the color transition at the end point to be indistinct.



12. Add 5 more drops of 5.0 N Sodium Hydroxide Standard Solution.

A pink color in the bottom (chloroform) layer at this point does not necessarily indicate lead is present. Only after adding the potassium cyanide in the next step will the presence of lead be confirmed by a pink color.

Dithizone method for powder pillows (continued)

13. Add 2 heaping 1.0 g scoops of potassium cyanide to the funnel. Stopper.

Shake vigorously until the potassium cyanide is all dissolved (about 15 seconds).

14. Wait one minute for the layers to separate. The bottom (chloroform) layer will be pink if lead is present.

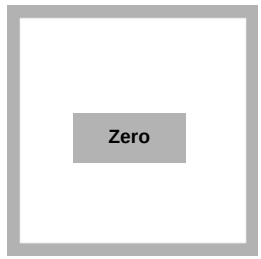
15. Prepared sample: Insert a cotton plug the size of a pea into the delivery tube of the funnel and slowly drain the bottom (chloroform) layer into a dry sample cell. Insert the stopper.

The lead-dithizone complex is stable for at least thirty minutes if the sample cell is kept tightly capped and out of direct sunlight.

16. Blank preparation: Measure 10 mL of chloroform into another sample cell.

Insert the stopper.

17. Insert the blank into the cell holder.

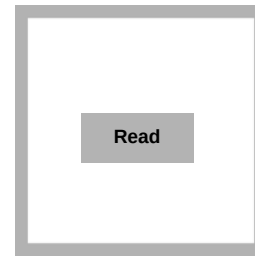


18. ZERO the instrument.

The display will show:

0 µg/L Pb^{2+}

19. Insert the prepared sample into the cell holder



20. READ the results in µg/L Pb^{2+}.

Interferences

Table 198 Substances that do not interfere

Non-interfering substance	Non-interfering substance
Aluminum	Lead
Antimony	Magnesium
Arsenic	Manganese
Calcium	Nickel
Chromium	Tin
Cobalt	Zinc
Iron	

Interference from the metals in the *Interfering substances* table can be eliminated by inserting the *Interference treatment for metals* procedure after step 6 of the *Dithizone method for powder pillows* procedure.

Table 199 Interfering substances

Interfering substance	Interference level
Highly buffered samples or extreme sample pH	All levels. See *Interference treatment for metals*.
Bismuth	All levels. See *Interference treatment for metals*.
Copper	All levels. See *Interference treatment for metals*.
Mercury	All levels. See *Interference treatment for metals*.
Silver	All levels. See *Interference treatment for metals*.
Tin	All levels. See *Interference treatment for metals*.

Interference treatment for metals

1. Measure about 5 mL of the DithiVer solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals. Draw off and collect the bottom (chloroform) layer for proper disposal.
2. Repeat extraction with fresh 5 mL portions of prepared dithizone solution (collecting the bottom layer each time in appropriate waste collection vessel) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated a number of times without appreciably affecting the amount of lead in the sample.
3. Extract the solution with several 2 or 3 mL portions of pure chloroform to remove any remaining dithizone, again collecting the bottom layer each time for proper disposal.
4. Continue the procedure, substituting 28.5 mL of prepared dithizone solution for the 30 mL in step 8.

DithiVer solution preparation, storage and reagent blank

- Store DithiVer Powder Pillows away from light and heat.
- A convenient way to prepare this solution is to add the contents of 10 DithiVer Metals Reagent Powder Pillows to a 500 mL bottle of chloroform.
- Invert several times until well mixed (carrier powder may not dissolve).
- Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.
- Carry out a reagent blank using deionized water through the entire method to obtain the most accurate results.

Sample collection, preservation and storage

- Collect samples in an acid-washed glass or plastic containers.
- Adjust the pH to 2 or less with nitric acid (about 2 mL per liter).
- Store preserved samples up to six months at room temperature.
- Adjust the pH to 2.5 with 5.0 N sodium hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Lead Voluette Ampule Standard, 50 mg/L Pb
- Ampule breaker
- TenSette Pipet and Pipet Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.Verify that units are in µg/L.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 250 mL portions of fresh sample.
6. Follow the *Dithizone method for powder pillows* test procedure for each of the spiked samples starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Lead Standard Solution, 100 mg/L
- Deionized water
- 100 mL Class A volumetric flask

- Class A volumetric pipet, 10 mL
- Pipet filler

1. Prepare a 10 mg/L lead standard solution as follows:
   a. Pipet 10.00 mL of Lead Standard, 100 mg/L, into a 100 mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
2. Prepare a 200 µg/L lead standard solution as follows:

   Use a graduated cylinder to measure 245 mL of deionized water into the 500 mL separatory funnel (step 3 of the *Dithizone method for powder pillows* test). Pipet 5.00 mL of the 10.0 mg/L Lead standard into the funnel.
3. Follow the *Dithizone method for powder pillows* test procedure.
4. To adjust the calibration curve using the reading obtained with the 200 µg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
280	150 µg/L Pb	140–160 µg/L Pb	2.3 µg/L

Summary of method

The dithizone method is designed for the determination of lead in water and wastewater. The DithiVer Metals Reagent is a stable powder form of dithizone. Lead ions in basic solution react with dithizone to form a pink to red lead-dithizonate complex, which is extracted with chloroform. Test results are measured at 515 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Lead Reagent Set (100 Tests)	—	—	2243100
Includes: (1) 1420299, (2) 1445817, (1) 1261699, (2) 76714, (1) 245053, (2) 245026			
Buffer Powder Pillows, citrate	1	100/pkg	1420299
Chloroform, ACS	30 mL	4 L	1445817
DithiVer Metals Reagent Powder Pillows	1	100/pkg	1261699
Potassium Cyanide	0.1 g	125 g	76714
Sodium Hydroxide Solution, 5.0 N	5 mL	1000 mL	245053
Sodium Hydroxide Standard Solution, 5.0 N	varies	59 mL DB	245026

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers, for opening powder pillows	1	each	96800
Cotton Balls, absorbent	1	100/pkg	257201
Cylinder, graduated, 5 mL	1	each	50837
Cylinder, graduated, 50 mL	1	each	50841
Cylinder, graduated, 250 mL	1	each	50846
Cylinder, graduated, mixing, 50 mL	1	each	189641
Funnel, separatory, 500 mL	1	each	52049
pH Meter, sens***ion***™1, portable, with electrode	1	each	5170010
Spoon, measuring,1 g	1	each	51000
Support Ring, 4"	1	each	58001
Support Ring Stand, 5" x 8" base	1	each	56300
Sample Cell, 1-inch square, w/stopper, matched pair	2	2/pkg	2612602

Recommended standards

Description	Unit	Catalog number
Lead Standard Solution, 100 mg/L Pb	100 mL	1261742
Lead Standard Solution, 10 mL Voluette Ampules, 50 mg/L Pb	16/pkg	1426210

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule Breaker Kit	each	2196800
Chloroform, ACS	500 mL	1445849
Filter Discs, glass, 47 mm	100/pkg	253000
Filter Holder, glass, for 47-mm filter	each	234000
Flask, Erlenmeyer, 500 mL	each	50549
Flask, filtering, 500 mL	each	54649
Flask, volumetric, Class A, 100 mL	each	1457442
Nitric Acid Solution, 1:1	500 mL	254049
Nitric Acid, ACS	500 mL	15249
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	39133
Pipet, serological, 2 mL	each	53236
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet, volumetric, 5.00 mL, Class A	each	1451537
Pipet, volumetric, 10.00 mL, Class A	each	1451538
Pipet Filler, safety bulb	each	1465100
Sulfuric Acid, 5.25 N	100 mL MDB	244932
Water, deionized	4 L	27256

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Edition 7

LeadTrak™ Fast Column Extraction Method

Method 8317

5 to 150 µg/L

Scope and Application: For drinking water

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 200 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
The sampling requirements for “first-draw” analysis are detailed in *Sample collection, preservation and storage*.
Reagents will stain the sample cells, rinse the cells with 1:1 nitric acid, LeadTrak followed by deionized water.

Collect the following items:

Description	Quantity
LeadTrak™ Reagent Set	1
Beaker, polypropylene, 150 mL	2
Beaker, polypropylene, 250 mL	1
Clamp, 2-prong extension, with clamp holder	1
Cylinder, graduated polypropylene, 25 mL	1
Cylinder, graduated polypropylene, 100 mL	1
Dropper, 0.5 and 1.0 mL marks	1
Sample Cells (see *Instrument-specific information*)	1
Support for Ring Stand	1

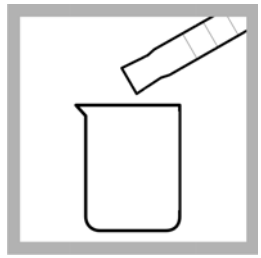
See *Consumables and replacement items* for reorder information.

LeadTrak Fast Column Extraction

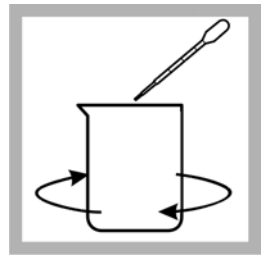
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a 100 mL plastic graduated cylinder with 100 mL of the sample. Pour the measured sample into a 250 mL plastic beaker.



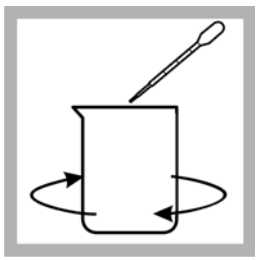
3. Using a plastic 1mL dropper, add 1.0 mL of pPb-1 Acid Preservative Solution to the sample and swirl to mix.

If the sample has been preserved previously with pPb-1 Acid Preservative at a ratio of 1.0 mL per 100 mL sample, omit steps 3 and 4.

Samples preserved with Nitric Acid require steps 3 and 4.

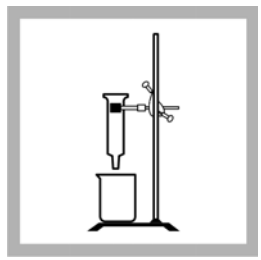
4. Start the instrument timer.

A two-minute reaction period will begin.



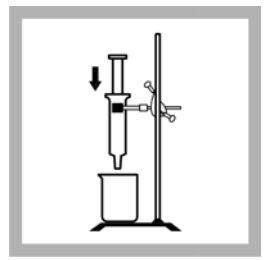
5. When the timer expires, use a second 1 mL plastic dropper to add 2.0 mL of pPb-2 Fixer Solution. Swirl to mix.

Field samples that have been preserved with nitric acid or samples that have been digested may exceed the buffer capacity of the Fixer Solution. After step 5, check the pH of these samples and adjust with 5 N Sodium Hydroxide to a pH of 6.7–7.1 before proceeding with step 6.



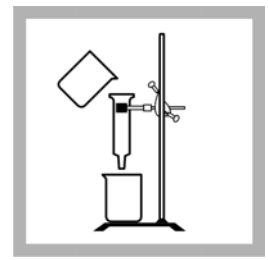
6. Mount a new Fast Column Extractor in a ring stand with a clamp. Place a 150-mL plastic beaker under the Extractor.

A Fast Column Extractor is included in the LeadTrak® Reagent Set. A new extractor is required for each test.



7. Soak the cotton plug with deionized water and compress it with the plunger. Remove the plunger. If the cotton plug moves up the column, push it back to the bottom with a clean, blunt rod.

The cotton plug should fit snugly against the inner wall of the column.

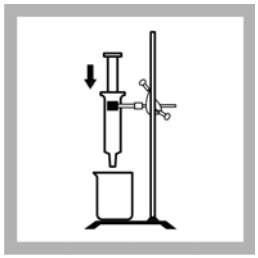


8. Pour the prepared sample slowly into the center of the Column Extractor. Wait for the sample to flow through.

The sample solution should flow relatively slowly (2 drops per second) through the column.

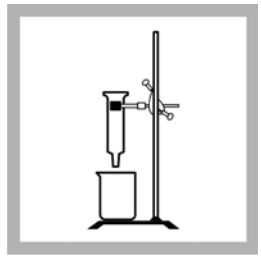
Keep the level of the sample solution just above the cotton plug.

LeadTrak Fast Column Extraction (continued)



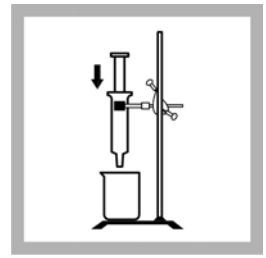
9. After the flow has stopped, fully compress the absorbent pad in the Extractor with the plunger. Discard the contents of the beaker. Slowly withdraw the plunger from the Extractor.

The absorbent pad should remain at the bottom of the Extractor when the plunger is removed. If the cotton plug moves up the column, push it back to the bottom with a clean, blunt rod.



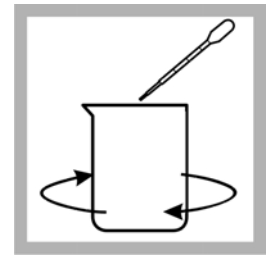
10. Place a clean, dry 150 mL beaker under the Extractor. Using a 25 mL plastic graduated cylinder, add 25 mL of pPb-3 Eluant Solution to the Extractor.

Keep the level of the eluent solution just above the absorbent pad.

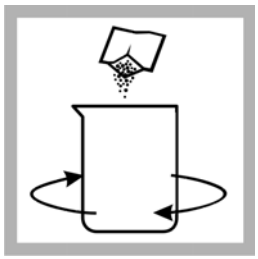


11. Allow the Eluant Solution to drip slowly from the Extractor.

After the flow has stopped, fully compress the absorbent pad.

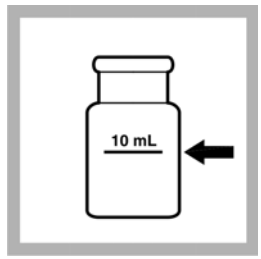


12. Using a 1 mL plastic dropper, add 1.0 mL of pPb-4 Neutralizer Solution to the beaker. Swirl thoroughly to mix and proceed immediately to step 13.



13. Add the contents of one pPb-5 Indicator Powder Pillow to the beaker and swirl thoroughly to mix.

The solution will turn brown.



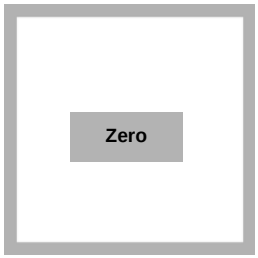
14. Pour 10 mL of solution into a sample cell.

15. Start the instrument timer.

A two-minute reaction period will begin.

16. When the timer expires, insert the sample cell.

LeadTrak Fast Column Extraction (continued)



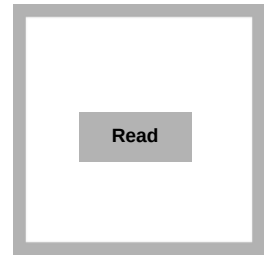
17. **ZERO** the instrument.

The display will show:

0 µg/L Pb

18. Remove the sample cell and add 3 drops of pPb-6 Decolorizer Solution to the cell. Swirl vigorously to mix

19. Insert the sample cell into the cell holder.



20. **READ** the results in µg/L Pb.

Interferences

Interference studies were conducted by preparing a known lead solution of 25 µg/L as well as the potential interfering ion. The ion was said to interfere when the resulting lead concentration changed by ± 10%. Samples containing levels exceeding these concentration values may be diluted 1:1 and analyzed. Multiply the value obtained by a factor of 2 to determine the lead present in the original sample.

To avoid contamination, do not use black rubber stoppers, black dropper bulbs and droppers with inked graduations. Use the plastic droppers provided in the reagent set.

Acid-wash all glassware and plasticware to prevent sample contamination, especially if the previous sample had a high lead level (see *Apparatus and sample preparation*).

The Extractor plunger may be reused for more than one test but should be rinsed with lead-free water between uses.

Table 201 Interfering substances

Interfering substance	Interference level
Aluminum, Al^{3+}	0.5 mg/L
Ammonium, NH_4^+	500 mg/L
Barium, Ba^{2+}	6 mg/L
Calcium, Ca^{2+}	500 mg/L
Chloride, Cl^-	1000 mg/L
Copper, Cu^{2+}	2 mg/L
Fluoride, F^-	10 mg/L
Iron, Fe^{2+}	2 mg/L
Magnesium, Mg^{2+}	500 mg/L
Manganese, Mn^{2+}	0.5 mg/L
Nitrate, NO_3^-	1000 mg/L
Sulfate, SO_4^{2-}	1000 mg/L
Zinc, Zn^{2+}	1 mg/L

Apparatus and sample preparation

Because lead is very common to our environment, care must be taken to prevent sample contamination. Follow these steps for greatest test accuracy:

- Lead-free water is necessary to minimize sample contamination when rinsing apparatus or diluting sample. The water may be either distilled or deionized. If the water is obtained from a grocery store, verify the lead concentration is zero from the label. If the lead concentration is uncertain, determine the lead concentration with the LeadTrak test.
- Plastic or glass sample containers and lids may be checked for contamination by rinsing with 1 mL of pPb-1 Acid Preservative Reagent*. Add 100 mL of lead-free water. After 24 hours, analyze this solution using the LeadTrak® test to confirm the absence of lead.
- Rinse glassware used in this test with a small amount of dilute lead-free 0.1 N nitric acid or pPb-1 Acid Preservative Reagent followed by rinsing with lead-free water.
- pPb-5 Indicator may be rinsed from the glass sample cells with a few drops of pPb-1 Acid Preservative Reagent or a small amount of dilute lead-free nitric acid.
- Acidify solutions containing lead with Nitric Acid or pPb-1 to below pH 2 to prevent adsorption of lead onto the container walls. See *Sample collection, preservation and storage*.

Sample collection, preservation and storage

- Samples may be collected either from household pipes (point-of-use) or from water sources.
- Preserved samples may be stored up to six months.
- Each sample type typically requires different sampling procedures. Consult with the appropriate regulatory agency for more information about specific sampling requirements.

Sampling for lead contamination in household pipes for point-of-use drinking water

- The sample should be collected after sitting in pipes with no flow for a minimum of six hours.
- Add 10 mL of pPb-1 Acid Preservative* to a one-liter bottle.
- Turn on tap and collect exactly the first liter of water in the bottle containing acid preservative.
- Cap and invert several times to mix.
- After two minutes the sample is ready for analysis. Steps 3 and 4 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in step 5.

Sampling for lead contamination from drinking water sources such as well water or water from main supply lines

- Add 10 mL of pPb-1 Acid Preservative* to a one-liter bottle.
- Turn on the tap for 3–5 minutes or until the water temperature has been stable for 3 minutes.
- Collect exactly one liter of water into the bottle containing the acid preservative.
- Cap and invert several times to mix.
- After two minutes the sample is ready for analysis. Steps 3 and 4 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in step 5.
- At least one liter should be collected to obtain a representative sample. If less than one liter is collected, use 1 mL of pPb-1 Acid Preservative per 100 mL of sample.
- If nitric acid is to be substituted for pPb-1 as a preservative or the sample is digested, the buffering capacity of the pPb-2 Fixer Solution* may be exceeded. Adjust the sample pH to 6.7–7.1 pH with 5 N Sodium Hydroxide* after step 6.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- 10 mg/L (10,000 µg/L) Lead Standard Solution
- TenSette Pipet and pipet tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 100 mL portions of fresh sample.
6. Follow the *LeadTrak Fast Column Extraction* test procedure for each of the spiked samples starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Lead Standard Solution, 1000 mg/L or Lead Voluette® Ampule Standard Solution, 50-mg/L as Pb
- Lead-free water or Deionized water
- 100 mL Class A volumetric flask or 100-mL plastic volumetric flask
- 1.0 mL Class A volumetric pipet
- TenSette Pipet and Pipet Tips

1. Prepare a 100 µg/L Lead standard solution as follows:
   a. Pipet 1.0 mL of Lead Standard, 1000 mg/L, into a 100 mL volumetric flask.
   b. Use a Tensette Pipet to add 0.2 mL of concentrated nitric acid to the flask
   c. Dilute to the mark with lead-free deionized water.
   d. Pipet 10.00 mL of this prepared solution into a 1 liter plastic volumetric flask.
   e. Add 2.0 mL of nitric acid to the flask.
   f. Dilute to the mark with lead-free water.
   g. Prepare this solution immediately before use.

   OR

1. Prepare a 100 µg/L Lead Standard Solution as follows:
   a. Use a Tensette Pipet to add 0.2 mL from a Lead Voluette® Ampule Standard Solution, 50 mg/L as Pb into a 100 mL plastic volumetric flask.
   b. DIlute to volume with deionized water. Prepare solution immediately before use

2. Use the standard solution in place of the sample. Follow the *LeadTrak Fast Column Extraction* test procedure.
3. To adjust the calibration curve using the reading obtained with the 100-mg/L Standard Solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Point of Curve	Concentration
283	50 µg/L Pb^{2+}	45–55 µg/L Pb^{2+}	Entire curve	4 µg/L Pb^{2+}

Summary of method

Acid soluble lead, as Pb^{2+}, in a potable water sample is first concentrated on a Fast Column Extractor. The lead is then eluted from the Extractor and determined colorimetrically with an indicator. Test results are measured at 477 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
LeadTrak™ Reagent Set	1	20/pkg	2375000

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, polypropylene, 150 mL	2	each	108044
Beaker, polypropylene, 250 mL	1	each	108046
Clamp, 2-prong extension	1	each	2114500
Clamp Holder	1	each	32600
Cylinder, graduated polypropylene, 25 mL	1	each	108140
Cylinder, graduated polypropylene, 100 mL	1	each	108142
Dropper, 0.5 and 1.0 mL marks	1	20/pkg	2124720
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Support for Ring Stand	1	each	56300

Recommended standards and apparatus

Description	Unit	Catalog number
Flask, volumetric, polypropylene, 1000 mL	each	2099553
Flask, volumetric, polypropylene, 100 mL	each	2099542
Lead Standard Solution, 1000 mg/L as Pb	100 mL	1279642
Lead Standard Solution, 50-mg/L 10 mL Voluette® Ampules	16/pkg	1426210
Lead Standard Solution, 10 mg/L	25 mL	2374820
Nitric Acid, ACS	500 mL	15249

Recommended standards and apparatus (continued)

Description	Unit	Catalog number
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, Class A, 1.00 mL	each	1451535
Pipet Filler, safety bulb	each	1465100
Pipet, volumetric, Class A, 10.00 mL	each	1451538
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
pPb-1 Acid Preservative Reagent	236 mL	2368531
pPb-2 Fixer Solution	43 mL	2368655
Sodium Hydroxide, 5.0 N	1 L	245053

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Edition 7

Manganese

DOC316.53.01058

USEPA[1] Periodate Oxidation Method[2] **Method 8034**

HR (0.1 to 20.0 mg/L) **Powder Pillows**

Scope and Application: For soluble manganese in water and wastewater

[1] USEPA Approved for reporting wastewater analyses (digestion required). *Federal Register*, 44(116)34 193 (June 14, 1979)

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 202 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Digestion is required for reporting wastewater analyses.
If only dissolved manganese is to be determined, filter the sample before acid addition.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

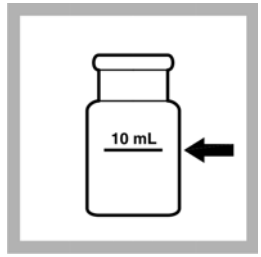
Description	Quantity
High Range Manganese Reagent Set	1
Sample Cells (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

Periodate Oxidation method for powder pillows

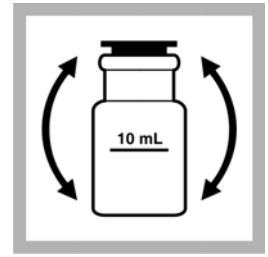
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Prepared Sample: Fill a sample cell with 10 mL of sample.

3. Add the contents of one Buffer Powder Pillow, Citrate Type for Manganese.



4. Stopper or cap and invert to mix.

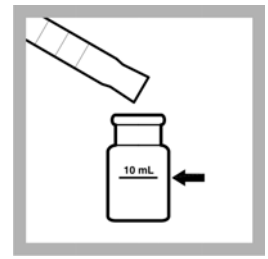
5. Add the contents of one Sodium Periodate Powder Pillow to the sample cell.

6. Stopper or cap and invert to mix.

A violet color will develop if manganese is present.

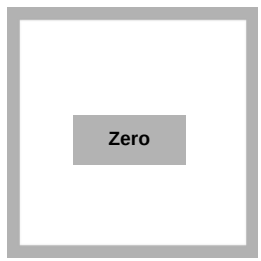
7. Start the instrument timer.

A two-minute reaction time will begin.



8. Blank Preparation: Fill a second sample cell with 10 mL of sample.

9. When the timer expires, insert the blank into the cell holder.

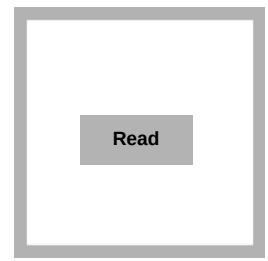


10. ZERO the instrument.

The display will show:

0.0 mg/L Mn

11. Within eight minutes after the timer expires, insert the sample into the cell holder



12. READ the results in mg/L Mn.

Interferences

Table 203 Interfering substances

Interfering substance	Interference level
Calcium	700 mg/L
Chloride	70,000 mg/L
Iron	5 mg/L
Magnesium	100,000 mg/L
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

- Collect samples in acid-washed plastic bottles. Do not use glass containers due to possible adsorption of Mn to glass.
- If samples are acidified, adjust the pH to 4–5 with 5.0 N Sodium Hydroxide before analysis.
- Do not exceed pH 5, as manganese may precipitate.
- Correct the test result for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Manganese Voluette® Ampule Standard, 250 mg/L Mn
- Ampule breaker
- TenSette Pipet

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard Additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample. Mix thoroughly.
6. Follow the *Periodate Oxidation method for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Manganese Standard Solution, 1000 mg/L
- Deionized water

- 1 L Class A volumetric flask
- Class A volumetric pipet, 10 mL
- Pipet filler, safety bulb

1. Prepare a 10.0 mg/L manganese standard solution as follows:
   a. Pipet 10.0 mL of Manganese Standard, 1000 mg/L, into a 1000 mL (1 liter) volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use this solution in place of the sample. Follow the *Periodate Oxidation method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, select Options>More>Standard Adjust from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Portion of Curve	Concentration
295	10.0 mg/L Mn	9.6–10.4 mg/L Mn	Entire curve	0.1 mg/L Mn

Summary of method

Manganese in the sample is oxidized to the purple permanganate state by sodium periodate, after buffering the sample with citrate. The purple color is directly proportional to the manganese concentration. Test results are measured at 525 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Manganese Reagent Set, High Range (100 tests), includes:	—	—	2430000
Buffer Powder Pillows, citrate type for Manganese	1	100/pkg	2107669
Sodium Periodate Powder Pillows for Manganese	1	100/pkg	2107769

Required apparatus

Description	Quantity	Unit	Catalog number
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stopper, rubber	1	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Manganese Standard Solution, 1000 mg/L Mn	100 mL	1279142
Manganese Standard Solution, 250 mg/L Mn, 10-mL Voluette® ampule	16/pkg	1425810
Water, deionized	4 L	27256
Voluette Ampule breaker	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Manganese Standard Solution, 2 mL PourRite® Ampule, 25 mg/L	20/pkg	2112820
Manganese Standard Solution, 2 mL PourRite® Ampule, 10 mg/L	20/pkg	2605820
pH paper, 0–14	100/pkg	2601300
Pipe filler, safety bulb	each	1465100
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, TenSette, 1.0–10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
Pipet Tips, for TenSette Pipet 1970010	50/pkg	2199796
PourRite® Ampule breaker	each	2484600
Sodium Hydroxide, 5.0 N	100 mL	245032
Volumetric flask, Class A, 1000 mL	each	1457453
Volumetric pipet, Class A, 10 mL	each	1451538

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Edition 7

Manganese

DOC316.53.01057

1-(2-Pyridylazo)-2-Naphthol PAN Method[1] **Method 8149**

LR (0.006 to 0.700 mg/L) **Powder Pillows**

Scope and Application: For water and wastewater; digestion is required for determining total manganese

[1] Adapted from Goto, K., et al., *Talanta*, 24, 652-3 (1977)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 204 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Rinse all glassware with 1:1 Nitric Acid Solution. Rinse again with deionized water.
The alkaline cyanide solution contains cyanide. Cyanide solutions should be collected for disposal as a reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. Refer to the current MSDS for safe handling and disposal instructions.
Total manganese determination requires a prior digestion. Refer to the *Water Analysis Guide* for more information.

Collect the following items:

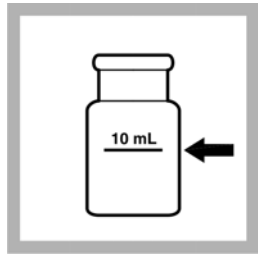
Description	Quantity
Alkaline Cyanide Reagent	12 drops
Ascorbic Acid Powder Pillows	2
PAN Indicator Solution, 0.1%	12 drops
Deionized Water	10 mL
Sample Cells (see *Instrument-specific information*)	2
Stoppers for 18 mm tube	2

See *Consumables and replacement items* for reorder information.

PAN method for powder pillows

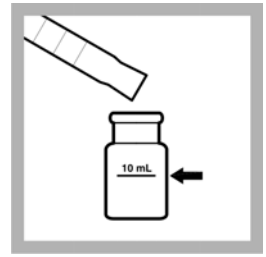
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Blank Preparation: Pour 10.0 mL of deionized water into a sample cell.

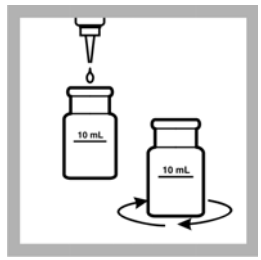
Total manganese determination requires prior digestion.



3. Prepared Sample: Pour 10.0 mL of sample into another sample cell.

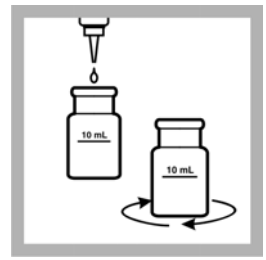
4. Add the contents of one Ascorbic Acid Powder Pillow to each cell.

5. Stopper and invert to dissolve the powder.



6. Add 12 drops of Alkaline-Cyanide Reagent Solution to each cell. Swirl gently to mix.

A cloudy solution may form. The turbidity should dissipate after step 7.



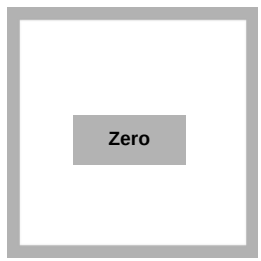
7. Add 12 drops of PAN Indicator Solution, 0.1%, to each sample cell. Swirl gently to mix.

An orange color will develop in the sample if manganese is present.

8. Start the instrument timer.

A two-minute reaction period will begin.

9. When the timer expires, wipe the blank and place it in the cell holder.

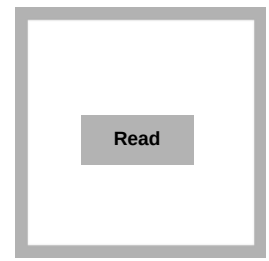


10. ZERO the instrument.

The display will show:

0.000 mg/L Mn

11. Wipe the prepared cell and place it in the holder.



12. READ the results in mg/L Mn.

Interferences

For samples that contain hardness greater than 300 mg/L $CaCO_3$, add 4 drops of Rochelle Salt Solution to the sample **after** adding the Ascorbic Acid Powder Pillow in step 4.

Table 205 Interfering substances

Interfering substance	Interference level
Aluminum	20 mg/L
Cadmium	10 mg/L
Calcium	1000 mg/L as $CaCO_3$
Cobalt	20 mg/L
Copper	50 mg/L
Iron	25 mg/L (If sample contains more than 5 mg/L iron, allow a 10-minute reaction period in step 8.)
Lead	0.5 mg/L
Magnesium	300 mg/L as $CaCO_3$
Nickel	40 mg/L
Zinc	15 mg/L

Sample collection, preservation and storage

- Collect samples in a clean plastic container.
- Adjust the pH to 2 or less with Concentrated Nitric Acid* (about 2 mL per liter).
- Preserved samples can be stored up to six months at room temperature.
- Adjust the pH to between 4–5 with 5.0 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Manganese PourRite® Ampule Standard, 10-mg/L Mn
- Mixing cylinders (3)
- Ampule breaker, PourRite
- TenSette Pipet

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select Options>More>Standard additions from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10 mL portions of fresh sample. Mix thoroughly.

6. Follow the *PAN method for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.

7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Manganese Voluette Standard Solution, 250 mg/L Mn
- 1 L Class A volumetric flask
- Deionized water
- Class A volumetric pipet, 2 mL
- Pipet filler, safety bulb

1. Prepare a 0.5 mg/L manganese standard solution as follows:

   a. Pipet 2.0 mL of Manganese Standard, 250 mg/L as Mn, into a 1000 mL (1 liter) volumetric flask.

   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.

2. Use this solution in place of the sample. Follow the *PAN method for powder pillows* test procedure.

3. To adjust the calibration curve using the reading obtained with the standard solution, select Options>More>Standard Adjust from the instrument menu.

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
290	0.500 mg/L Mn	0.491–0.509 mg/L Mn	0.006 mg/L Mn

Summary of method

The PAN method is a highly sensitive and rapid procedure for detecting low levels of manganese. An ascorbic acid reagent is used initially to reduce all oxidized forms of manganese to Mn^{2+}. An alkaline-cyanide reagent is added to mask any potential interferences. PAN Indicator is then added to combine with the Mn^{2+} to form an orange-colored complex. Test results are measured at 560 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Manganese Reagent Set, 10 mL (50 tests), includes:	—	—	2651700
Alkaline Cyanide Reagent	12 drops	50 mL SCDB	2122326
Ascorbic Acid Powder Pillows	2 pillows	100/pkg	1457799
PAN Indicator Solution, 0.1%	12 drops	50 mL SCDB	2122426
Water, deionized	10 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stoppers for 18 mm Tube	2	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Manganese Standard Solution, 10-mg/L Mn, 2 mL PourRite® ampule	20/pkg	2605820
Manganese Standard Solution, 250-mg/L Mn, 10-mL Voluette® ampule	16/pkg	1425810
Voluette Ampule breaker 10 mL	each	2196800
PourRite® Ampule breaker 2 mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25 mL	each	2088640
Nitric Acid, concentrated	500 mL	15249
pH paper, 0-14	100/pkg	2601300
Pipet Filler, safety bulb	each	1465100
Pipet, TenSette® 0.1–1.0 mL	each	1970001
Pipet, TenSette, 1.0–10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet Tips, for TenSette Pipet 1970010	50/pkg	2199796
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
Rochelle Salt Solution	29 mL	172533
Sodium Hydroxide, 5.0 N	100 mL	245032
Stopper for 18 mm tube	25/pkg	173125
Volumetric flask, Class A, 1000 mL	each	1457453
Volumetric pipet, Class A, 2 mL	each	1451536
PourRite® Ampule breaker 2 mL	each	2484600
Manganese Standard Solution, 2-mL PourRite® Ampule, 25 mg/L	20/pkg	2112820

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Edition 7

Cold Vapor Mercury Concentration Method[1] Method 10065

0.1 to 2.5 µg/L Hg

Scope and Application: For water, wastewater and seawater

[1] Patent number 5,733,786

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 206 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Perform phase 1 of the procedure in a fume hood. Toxic chlorine or other gases may be produced.
Use dedicated digestion glassware and sample cells for this procedure.
Determine a reagent blank for each new lot of reagent by running the entire procedure, including the digestion, using one liter of deionized water instead of sample. Add the same amount of potassium permanganate as required by the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

Description	Quantity
Cold Vapor Mercury Reagent Set (see *Required apparatus* for contents of reagent set)	1
Digestion Reagents and Apparatus (see *Required digestion reagents and apparatus*)	varies
Cold Vapor Mercury Apparatus Set	1
Sample Cells, 1-inch square, 10-mL, matched pair (wherever applicable)	2
See *Required apparatus* for a complete list of required apparatus.	

See *Consumables and replacement items* for reorder information.

Phase 1: Sample digestion

DANGER
Toxic gas hazard. Test must be performed under a fume hood.

1. Transfer one liter of the sample to a 2000 mL Erlenmeyer flask. Add a 50-mm magnetic stir bar to the sample. Set the flask on a magnetic stirring hot plate and begin stirring.

2. Add 50 mL of concentrated sulfuric acid to the sample.

3. Add 25 mL of concentrated nitric acid to the sample.

4. Add 4.0 g of potassium persulfate to the sample. Stir until dissolved.

Alternatively, add one 5 gram measuring scoop of potassium persulfate to the sample.

5. Add 7.5 g of potassium permanganate to the sample. Stir until dissolved.

Alternatively, add a 10 gram measuring scoop of potassium permanganate to the sample.

6. Cover the flask with a watch glass. Begin heating the sample to a temperature of 90 °C after the reagents have dissolved. **Do not boil.**

For a mercury standard or reagent blank in distilled water, the heating step is not necessary.

7. Continue to stir and heat the sample at 90 °C for two hours.

The solution must remain dark purple throughout the entire digestion. Some samples, such as sea waters, industrial effluents or other samples high in organic matter or chloride concentration, require additional permanganate. It may be difficult to see a dark purple color if the sample contains black/brown manganese dioxide precipitate. Add more potassium permanganate if the solution is not dark purple.

8. Cool the digested sample to room temperature. Turn the hot plate off.

A brown/black precipitate of manganese dioxide may settle during cooling. If the digested sample does not have a purple color, the digestion may be incomplete. Add more potassium permanganate. Return the sample to the magnetic stirring hot plate and continue the digestion until the purple color persists.

Phase 1: Sample digestion (continued)

DANGER
Toxic gas hazard. Test must be performed under a fume hood.

9. Return the cool, digested sample to the cool, magnetic stirring hot plate. Turn on the stirrer.

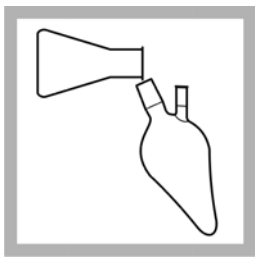
10. Using a 0.5 g measuring spoon, add 0.5 g additions of hydroxylamine-hydrochloride until the purple color disappears. Wait 30 seconds after each addition to see if the purple disappears. Add hydroxylamine-hydrochloride until all manganese dioxide is dissolved.

11. Remove the stir bar.

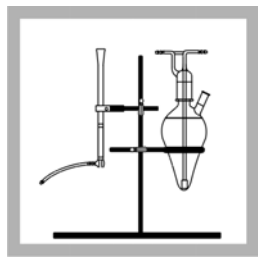
12. The digested sample is now ready for processing by cold vapor separation and preconcentration. Proceed to Phase 2.

Phase 2: Cold vapor separation and preconcentration of mercury

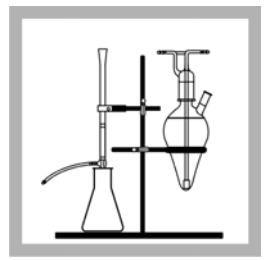
DANGER
Toxic gas hazard. Test must be performed under a fume hood.



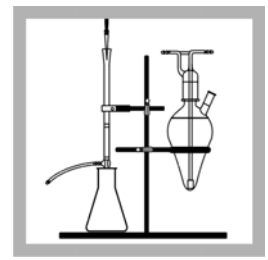
1. Transfer the digested sample to the Cold Vapor Gas Washing Bottle. (The volume of the digested sample should contain 0.1 to 2.5 µg Hg.)



2. Set the Gas Washing Bottle in the support ring. Place the top on the Gas Washing Bottle. Wait until step 9 to connect the mercury absorber column to the Gas Washing Bottle.



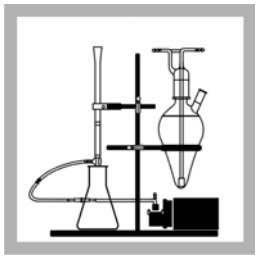
3. Connect the 100 mL Erlenmeyer flask to the mercury absorber column.



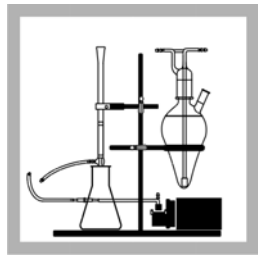
4. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber column.

Phase 2: Cold vapor separation and preconcentration of mercury (continued)

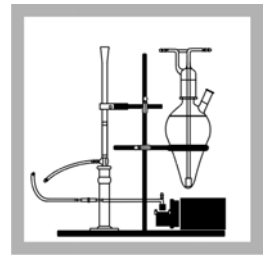
DANGER
Toxic gas hazard. Test must be performed under a fume hood.



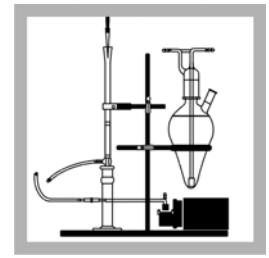
5. Connect the power to the vacuum pump and apply vacuum to the Mercury Absorber Column. Draw most of the HgEx Reagent B into the Erlenmeyer flask.



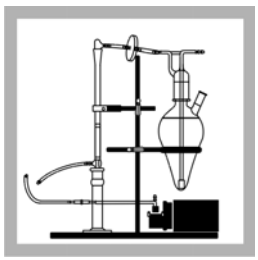
6. Disconnect the vacuum using the quick disconnect when HgEx Reagent B begins to drip from the inner delivery tube on the Mercury Absorber Column (about 10 seconds after starting the vacuum). Do not draw enough air through the column to begin drying the packing.



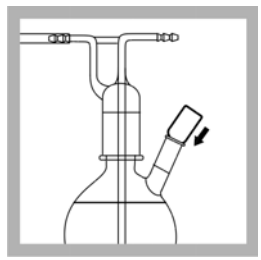
7. Remove the 100 mL Erlenmeyer flask from the Mercury Absorber Column. Replace it with the 10 mL Distilling Receiver.



8. Pipet 2 mL of HgEx Reagent C into the Mercury Absorber Column.

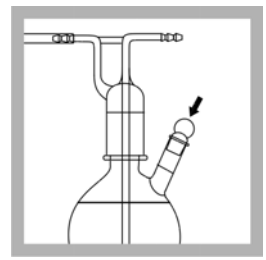


9. Connect the Mercury Absorber column to the Gas Washing Bottle using the glass elbow.

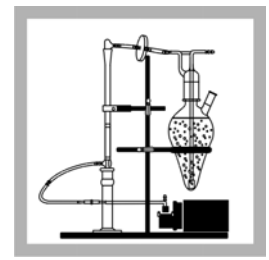


10. Shake an ampule of HgEx Reagent A to suspend undissolved reagent.

Open the ampule and gently shake the contents into the Gas Washing Bottle through the side neck.



11. Stopper the side neck on the Glass Washing Bottle.



12. Reconnect the vacuum to the Mercury Absorber Column using the quick disconnect. The vacuum will pull HgEx Reagent C through the Mercury Absorber Column packing and into the 10 mL receiver. Air bubbles should be produced at the gas dispersion tube in the Gas Washing Bottle. Perform steps 13–14 immediately.

Phase 2: Cold vapor separation and preconcentration of mercury (continued)

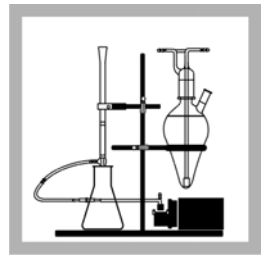
DANGER
Toxic gas hazard. Test must be performed under a fume hood.

13. Select the test.

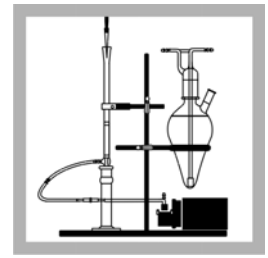
14. Start the instrument timer.

A five-minute reaction period will begin. Let the solution bubble for this period.

Air flow rate through the Gas Washing Bottle should be between 1–5 L/min. Allow more bubbling time for lower air flow rates. For example, if the air flow rate is 1 L/min., let the solution bubble for 10 minutes.



15. After the timer expires, remove the glass elbow from the top of the Mercury Absorber Column. Keep the vacuum pump on.



16. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber Column to elute the captured mercury.

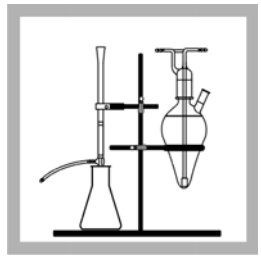
Continue to apply vacuum to pull the HgEx Reagent B into the Distilling Receiver.

Phase 2: Cold vapor separation and preconcentration of mercury (continued)

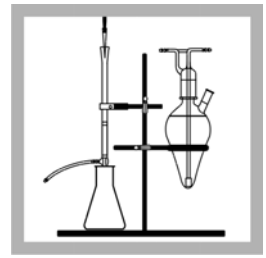
DANGER
Toxic gas hazard. Test must be performed under a fume hood.

17. Turn off or disconnect power to the vacuum pump when the volume in the Distilling Receiver reaches the 10 mL mark.

If necessary, the volume in the Distilling Receiver may be brought up to 10 mL with HgEx Reagent B. To avoid low volumes in the future, disconnect the vacuum a little sooner in step 6. This leaves more HgEx Reagent B in the packing of the Mercury Absorber Column.



18. Remove the distilling Receiver from the Mercury Absorber Column. Reconnect the 100-mL Erlenmeyer flask to the column.



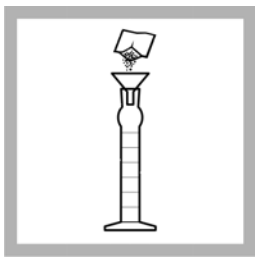
19. Pipet 3 mL of HgEx Reagent B into the Mercury Absorber Column without applying vacuum. This keeps the absorber packing wet between tests.

The Mercury Absorber Column eluate in the Distilling Receiver is ready for analysis.

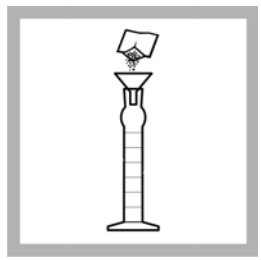
Proceed to Phase 3.

Phase 3: Colorimetric analysis

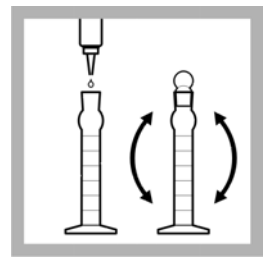
DANGER
Toxic gas hazard. Test must be performed under a fume hood.



1. Using the funnel provided, add the contents of one HgEx Reagent 3 foil pillow to the eluate in the Distilling Receiver. Stopper the receiver. Invert to dissolve the reagent.



2. Add the contents of one HgEx Reagent 4 foil pillow to the Distilling Receiver using the funnel provided. Stopper the receiver. Invert to dissolve the reagent.



3. Add 8 drops of HgEx Reagent 5 to the Distilling Receiver. Stopper the Receiver. Invert to mix.

4. Start the instrument timer.

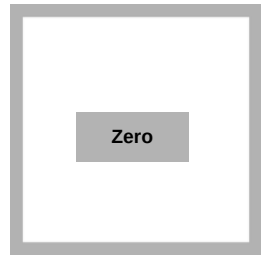
A two-minute reaction period will begin.

Phase 3: Colorimetric analysis (continued)

DANGER
Toxic gas hazard. Test must be performed under a fume hood.

5. During the reaction period, transfer the solution to a sample cell.

6. Wipe the sample cell. After the timer expires, insert the sample into the cell holder.



7. **ZERO** the instrument.

The display will show:

0.1 µg/L Hg (this program uses a non-zero intercept)

8. Remove the cell from the cell holder. Add the contents of one HgEx Reagent 6 foil pillow to the solution. Swirl the cell until the reagent is completely dissolved. Immediately go to step 9.

Do not use the funnel to add HgEx Reagent 6 to the sample cell. Any HgEx Reagent 6 in the funnel will make mercury undetectable in subsequent tests.

9. Return the sample cell to the cell holder.

10. **READ** the results in µg/L Hg. This is the concentration in the original sample.

Interferences

Standards were used to prepare a single test solution with substances at the concentrations listed in the *Interfering substances and levels* table. A second test solution containing only mercury at the same concentration was prepared as the control. The two solutions were digested, then analyzed concurrently. There was no interference from the matrix of the test solution at the concentrations listed.

In addition, no interference occurred with a test solution containing 1000 mg/L Na^{+}, 1000 mg/L K^{+}, 1000 mg/L Mg^{2+} and 400 mg/L Ca^{2+}.

Table 207 Interfering substances and levels

Interfering substance	Interference level
Ag^{+}	7 mg/L Ag^{+}
Al^{+3}	10 mg/L Al^{+3}
Au^{+3}	500 µg/L Au^{+3}
Cd^{+2}	10 mg/ L Cd^{+2}
Co^{+2}	10 mg/L Co^{+2}
Cr^{+6}	10 mg/L Cr^{+6}
Cu^{+2}	10 mg/L Cu^{+2}
F^{-}	1.0 mg/L F^{-}
Fe^{+2}	100 mg/L Fe^{+2}
Hg^{+2}	1 µg/L Hg^{+2}
Mo^{+6}	10 mg/L Mo^{+6}
Ni^{+2}	10 mg/L Ni^{+2}
NO_3^{-}–N	50 mg/L NO_3^{-}–N
Pb^{2+}	10 mg/L Pb^{2+}
SiO_2	100 mg/L SiO_2
Zn^{+2}	10 mg/L Zn^{+2}

Sample collection, preservation and storage

- Collect 1000 mL of sample in an analytically clean, glass or polyethylene terephthalate (PET) container.
- Add 10 mL of concentrated hydrochloric acid to preserve the sample before sample collection. Fill the container completely full to minimize air space when closed.

  Note: *Close a glass container with a ground glass stopper. Close a PET container with a PET cap or a polypropylene cap (no liner).*

- Store aqueous samples at 2–6 °C. Acid-preserved samples are stable for at least 6 months.

Accuracy check

Standard additions method

1. Prepare a 10.0 mg/L Mercury Standard Solution as described under *Standard solution method*, step 3a.
2. Use a TenSette® Pipet to add 0.10 mL of the 10.0 mg/L Mercury Standard Solution to the purged solution in the Gas Washing Bottle after an analysis has been performed. Immediately stopper the Gas Washing Bottle.
3. Begin at step 3 of Phase 2. Follow the procedure steps.
4. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

Standard solution method

1. Transfer 800 mL of deionized water into the Gas Washing Bottle.

2. Add 50 mL of concentrated sulfuric acid and 25 mL of concentrated nitric acid to the water. Swirl to mix.
3. Prepare a 0.1 mg/L mercury standard solution by serially diluting a 1000 mg/L Mercury Standard Solution:
   a. To make a 10.0 mg/L standard, add 1.0 mL of concentrated nitric acid to a 500 mL volumetric flask. Dilute 5.00 mL of a 1000 mg/L standard to 500 mL with deionized water. Mix well.
   b. To make a 1.0 mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100 mL volumetric flask. Dilute 10.0 mL of the 10.0 mg/L standard to 100 mL with deionized water. Mix well.
   c. To make a 0.1 mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100 mL volumetric flask. Dilute 10.00 mL of the 1.0 mg/L solution to 100 mL with deionized water. Mix well.
4. Pipet 10.0 mL of the 0.1 mg/L mercury standard solution into the Gas Washing Bottle. Swirl to mix.
5. Begin at step 2 of Phase 2. Follow the procedure steps.
6. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

System start up

For more accurate results, perform a few analyses on mercury standards and blanks for system equilibration before beginning sample testing. This allows the system to stabilize before processing samples.

Startup standard

1. Test a mercury standard solution by following the procedure under *Accuracy check* using the *Standard solution method*. Continue with step 2 of the Startup Standard procedure if the value is not within specified limits.
2. Pipet 10.0 mL of the 0.1 mg/L mercury standard solution into the purged solution in the Gas Washing Bottle. Immediately stopper the Gas Washing Bottle.
3. Begin at step 3 of Phase 2. Follow the procedure steps.
4. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg. Repeat steps 1–3 if the value is not within these limits.

Startup blank

Run a system blank by using the purged solution in the Gas Washing Bottle after a satisfactory test of the Startup Standard has been completed.

1. Leave the purged solution in the Gas Washing Bottle. Do not add an aliquot of mercury standard.
2. Begin at step 3 of Phase 2. Follow the procedure steps.
3. Test the eluate as described in Phase 3. The displayed concentration should be ≤ 0.2 µg/L Hg. Repeat the Startup Blank procedure until a reproducible value is obtained.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
312	1.0 µg/L Hg	0.9–1.1 µg/L Hg	0.03 µg/L Hg

Storage and maintenance of the cold vapor mercury apparatus

Storage
Store the apparatus as follows for fastest system stabilization and greatest sensitivity:

- Store the Gas Washing Bottle filled with deionized water containing 15 mL of concentrated sulfuric acid. Seal the bottle with the Gas Washing Bottle stopper and top.
- Store the Mercury Absorber Column with the packing wetted with HgEx Reagent B. The Erlenmeyer flask should be kept attached underneath the column. The top of the Mercury Absorber column should be attached to the Gas Washing Bottle with the glass elbow as in the procedure.

Glassware care
Use of dedicated glassware and sample cells is recommended because of the sensitivity of this procedure. Thoroughly clean the glassware and sample cells between tests. After washing, rinse with 1:1 hydrochloric acid solution, then rinse several times with deionized water.

Maintaining the system

- With proper care and storage, the Mercury Absorber Column may be used an unlimited number of times.
- Replace the Mercury Scrubber in the air trap housing at least once for every reagent set used.
- Moisture build up on the Gas Washing Bottle side of the Acro 50 Vent Filter will reduce the purging air flow rate. If this occurs replace the filter or dry it in an oven at 110 °C.

Summary of method

The sample is digested to convert all forms of mercury in the sample to mercuric (Hg^{2+}) ions. The mercuric ions in the digested sample are converted to mercury vapor in a semi-closed system. The vapor is carried by ambient air into a chemically activated absorber column where the mercury vapor is converted to mercuric chloride.

The mercuric chloride is eluted off the column and a sensitive indicator is added. The instrument is zeroed using the absorbance peak of the unreacted indicator. A complexing agent is added to break the mercury:indicator complex. The increase in unreacted indicator causes an increase in absorbance proportional to the amount of mercury in the original sample. Test results are measured at 412 nm.

Safety

Wear personal protective equipment such as safety glasses with side shields or a face shield to protect your eyes. Use other protective equipment as necessary (such as a fume hood) to avoid chemical exposure. Perform all steps exactly as prescribed in the procedure.

Pollution prevention and waste management

Proper management and disposal of waste is the responsibility of the waste generator. It is up to the generator to arrange for proper disposal and comply with applicable local, state and federal

regulations governing waste disposal. The manufacturer makes no guarantees or warranties, express or implied, for the waste disposal information represented in this procedure.

1. Dispose of the solution in the Gas Washing Bottle by neutralizing the solution to a pH of 6–9 and flushing to the sanitary sewer with water for several minutes.
2. The mercury contained in one liter of sample is concentrated by a factor of 100 by the Mercury Absorber Column. Mercury analysis within the range of the test may produce a solution in the sample cell that is above the RCRA Toxicity Characteristic limit of 0.20 mg/L Hg or other regulatory limits. The sample cell will contain 0.25 mg/L mercury if the original sample was at 2.5 µg/L mercury (the upper limit of the test range). Dispose of the solution in the sample cell according to applicable regulations.
3. The mercury scrubber will capture mercury vapor if the Mercury Absorber Column is not properly activated using HgEx Reagent B and HgEx Reagent C. In addition, mercury is also captured if the capacity of the Absorber Column is exceeded. If the Mercury Scrubber has captured mercury vapor, it must be disposed of according to applicable regulations.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Cold Vapor Mercury Reagent Set (25 tests), includes:			2658300
HgEx™ Reagent A, Stannous Sulfate Solution, 20 mL ampules	1	25/pkg	2658825
HgEx™ Reagent B, Sulfuric Acid Solution	19 mL	500 mL	2658949
HgEx™ Reagent C, Sodium Hypochlorite Solution	2 mL	55 mL	2659059
HgEx™ Reagent 3, Alkaline Reagent Powder Pillows	1 pillow	25/pkg	2658448
HgEx™ Reagent 4, Indicator Powder Pillows	1 pillow	25/pkg	2658548
HgEx™ Reagent 5, Sodium Hydroxide Solution	8 drops	10 mL SCDB	2658636
HgEx™ Reagent 6, Complexing Reagent Powder Pillows	1 pillow	25/pkg	2658748
Mercury Scrubber	2/reagent set	2/pkg	2655800

Required apparatus

Description	Quantity	Unit	Catalog number
Cold Vapor Mercury Apparatus Set, includes:			2674400
Acro 50 Vent Filter	1	18/pkg	2683318
Air Trap Holder Assembly	1	each	2663900
Ampule Breaker	1	each	2564000
Breaker/Capper Tool for Mercury Scrubber	1	each	2664000
C-flex Tubing, 0.25-inch ID, white	4 ft	25 ft	2327367
Clamp for Mercury Absorber Column	1	each	2656200
Clamp Holder	2	each	32600
Sample cell	10 mL Matched	2/pkg	2495402
Riser Cell, 1” Square DR2000/2010	1	each	4528200
Riser cell, 1”, Square DR3000	1	each	4840300
Distilling Receiver, 10-mL	1	each	2655438
Flask, Erlenmeyer, 100-mL	1	each	2655342

Required apparatus (continued)

Description	Quantity	Unit	Catalog number
Funnel, micro	1	each	2584335
Gas Washing Bottle, 1200-mL	1	each	2662200
Glass Elbow, 90-degree, with hose adapter	1	each	2655200
Mercury Absorber Column	1	each	2655510
Support Ring for Gas Washing Bottle	1	each	2656300
Stopper, for Distilling Receiver	1	each	2655900
Stopper, for Gas Washing Bottle	1	each	2662300
Support, Base and Rod	1	each	32900
Tubing Quick Disconnect, HDPE	1	12/pkg	1481000
Cylinder, graduated, 50-mL	1	each	50841
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet, TenSette, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 1970001	varies	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970010	varies	50/pkg	2199796
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Vacuum Pump, 115 VAC w/ North American Plug	1	each	2824800
Vacuum Pump, 230 VAC w/ North American Plug	1	each	2824801
Vacuum Pump, 230 V w/ European Plug	1	each	2824802

Required digestion reagents and apparatus

Description	Quantity	Unit	Catalog number
Flask, Erlenmeyer, 2000 mL	1	each	2489454
Hot Plate/Stirrer, 7 x 7, Cimarec Digital, 115 VAC	1	each	2881600
Digital Hot Plate 7 x 7, 230 VAC	1	each	2881502
Hydroxylamine Hydrochloride, ACS	varies	113 g	24614
Nitric Acid, ACS	25 mL	500 mL	15249
Potassium Permanganate, ACS	varies	454 g	16801H
Potassium Persulfate, ACS	4.0 g	454 g	2617501
Sulfuric Acid, ACS, concentrated	50 mL	2.5 L	97909
Spoon, measuring, 0.5 g	1	each	90700
Stir Bar, Octagonal 50.8 x 7.9 mm	1	each	2095355
Thermometer, –20 to 110 °C	1	each	56601
Watch Glass, Pyrex, 65 mm	1	each	57867

Recommended standards

Description	Unit	Catalog number
Mercury Standard Solution, 1000 mg/L Hg (NIST)	100 mL	1419542
Water, deionized	4 L	27256

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Edition 7

Molybdenum

DOC316.53.01061

Mercaptoacetic Acid Method[1] **Method 8036**

HR (0.2 to 40.0 mg/L Mo) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater, boiler and cooling waters

[1] Adapted from *Analytical Chemistry. 25(9) 1363 (1953)*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 208 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank from the result or adjust a reagent blank.
Filter turbid samples using filter paper and a funnel.
After all reagents have been added, the presence of molybdenum will cause a yellow color to form.
Adjust pH of acidified stored samples to pH 7 before analysis.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
MolyVer® 1 Molybdenum Reagent Powder Pillows	1
MolyVer® 2 Molybdenum Reagent Powder Pillows	1
MolyVer® 3 Molybdenum Reagent Powder Pillows	1
Sample Cell for blank (see *Instrument-specific information*)	2

Collect the following items: (continued)

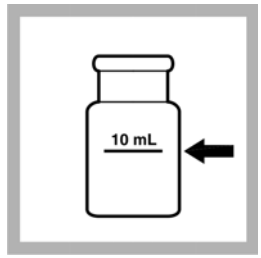
Description	Quantity
AccuVac Test:	
CDTA Solution, 0.4 M	4 drops
MolyVer® 6 Reagent AccuVac® Ampuls	1
Beaker, 50 mL	1
Sample Cells (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

Mercaptoacetic Acid for powder pillows

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Fill a sample cell with 10-mL of sample.

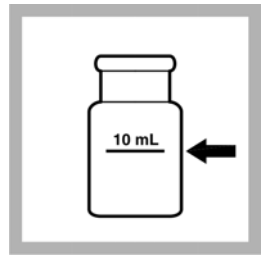
3. Prepared Sample: Add the contents of one MolyVer® 1 Reagent Powder Pillow. Swirl to mix.

4. Add the contents of one MolyVer 2 Reagent Powder Pillow. Swirl to mix.

5. Add the contents of one MolyVer 3 Reagent Powder Pillow. Swirl to mix.

6. Start the instrument timer.

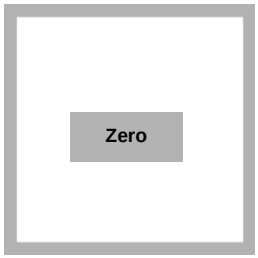
A five-minute reaction period will begin.



7. Blank Preparation: When the timer expires, fill a second sample cell with 10 mL of the original sample.

8. Insert the blank into the cell holder

Mercaptoacetic Acid for powder pillows (continued)

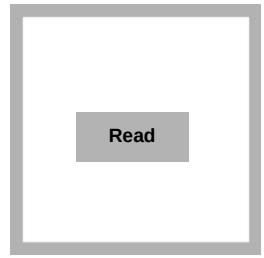


9. **ZERO** the instrument.

The display will show:

0.0 mg/L Mo^{6+}

10. Insert the prepared sample into the cell holder

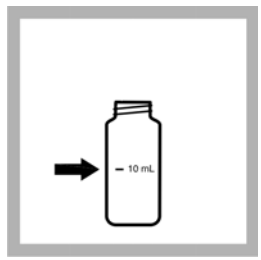


11. **READ** the results in mg/L Mo^{6+}.

Mercaptoacetic Acid for AccuVac® Ampuls

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. **Blank Preparation:** Fill a sample cell with 10 mL of sample.

3. **Prepared Sample:** Collect 40 mL of sample in a 50-mL beaker. Add four drops of 0.4 M CDTA Standard Solution to the sample in the beaker. Swirl to mix

4. Fill a MolyVer 6 AccuVac® Ampul with the treated sample. Make sure that the tip is immersed when filling the Ampul.

5. Quickly invert the Ampul several times to mix.

6. Start the instrument timer.

A five-minute reaction period will begin.

7. When the timer expired insert the blank into the cell holder.

ZERO the instrument.

The display will show:

0.0 mg/L Mo^{6+}

8. Insert the prepared sample into the cell holder.

READ the results in mg/L Mo^{6+}

Interferences

Table 209 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 50 mg/L
Chromium	Greater than 1000 mg/L
Copper	Samples containing 10 mg/L copper or more will exhibit an increasing positive interference upon standing. Read these samples as soon as possible after the five minute reaction period is complete.
Iron	Greater than 50 mg/L
Nickel	Greater than 50 mg/L
Nitrite	Interference from up to 2000 mg/L as NO_2^- can be eliminated by adding one Sulfamic Acid Powder Pillow[1] to the sample.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Adjust the pH to 2 or less with nitric acid (about 2 mL/L).
- Preserved samples can be stored up to 6 months at room temperature.
- Adjust the pH to 7 with 5.0 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

Standard additions method (sample spike) for powder pillows
Required for accuracy check:

- Molybdenum Standard Solution, 1000-mg/L Mo^{6+}
- Mixing cylinders (3)
- TenSette Pipet, 0.1 to 1.0 mL and Pipet tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **Options>More>Standard Additions** from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution.
5. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL and 0.6 mL of standard to three 30-mL portions of fresh sample. Mix thoroughly.
6. Follow the *Mercaptoacetic Acid for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)

1. Fill three 100 mL mixing cylinders each with 60-mL of sample and spike with 0.4 mL, 0.8 mL and 1.2 mL of standard.
2. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
3. Analyze each standard addition sample as described in the *Mercaptoacetic Acid for AccuVac® Ampuls*.
4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Molybdenum Standard Solution,10.0-mg/L Mo^{6+}

1. Use the molybdenum standard solution in place of the sample. Follow the *Mercaptoacetic Acid for powder pillows* or the *Mercaptoacetic Acid for AccuVac® Ampuls* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
320	10.0 mg/L Mo^{6+}	9.7–10.3 mg/L Mo^{6+}	0.2 mg/L Mo^{6+}
322	10.0 mg/L Mo^{6+}	9.7–10.3 mg/L Mo^{6+}	0.2 mg/L Mo^{6+}

Summary of method

MolyVer 1 and 2 Reagents are added to buffer and condition the sample. MolyVer 3 provides the mercaptoacetic acid which reacts with molybdate molybdenum to form a yellow color proportional to the molybdenum concentration. Test results are measured at 420 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Molybdenum Reagent Set, for 10-mL samples (100 tests):	—	—	2604100
MolyVer® 1 Molybdenum Reagent Powder Pillows	1	100/pkg	2604299
MolyVer® 2 Molybdenum Reagent Powder Pillows	1	100/pkg	2604399
MolyVer® 3 Molybdenum Reagent Powder Pillows	1	100/pkg	2604499
OR			
MolyVer® 6 Reagent AccuVac® Ampuls	1	25/pkg	2522025
CDTA Solution, 0.4 M	4 drops	15 mL SCDB	2615436

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Molybdenum Standard Solution, 10-mg-L as Mo	100 mL	1418742
Molybdenum Standard Solution, 1000-mg-L as Mo	100 mL	1418642
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Beakers, 50 mL	each	50041H
Cylinder, mixing, 50 mL	each	189641
Filter Paper, folded, 12.5 cm	100/pkg	189457
Funnel, poly, 65 mm	each	108367
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Sulfamic Acid Powder Pillows	100/pkg	105599
Cylinder, mixing, 100 mL	each	2088642

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Edition 7

Molybdenum

DOC316.53.01062

Ternary Complex Method **Method 8169**

LR (0.02 to 3.00 mg/L Mo) **Powder Pillows**

Scope and Application: For boiler and cooling tower waters

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 210 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Analyze samples immediately after collection.
Filter turbid samples using filter paper[1] and a funnel[1].

[1] See *Optional reagents and apparatus*.

Description	Quantity
Molybdenum Reagent Set for 20-mL sample	
Molybdenum 1 Reagent (LR) Molybdate Powder Pillow	1
Molybdenum 2 Reagent Solution	0.5 mL
Cylinder, graduated mixing, 25-mL	1
Sample Cells (see *Instrument-specific information*)	2

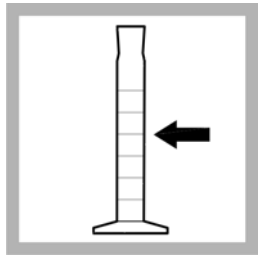
See *Consumables and replacement items* for reorder information.

Ternary Complex Method for powder pillows

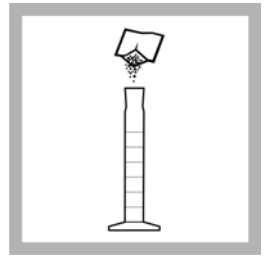
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

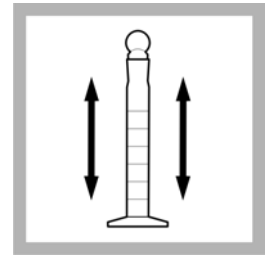
Refer to the user manual for orientation.



2. Fill a 25-mL graduated mixing cylinder with 20 mL of sample.



3. Add the contents of one Molybdenum 1 Reagent Powder Pillow to the graduated cylinder.



4. Prepared Sample: Stopper the cylinder and shake to completely dissolve the reagent.

5. Pour 10-mL of the prepared sample into a sample cell.

6. Developed Sample: Add 0.5 mL of Molybdenum 2 Reagent to the sample cell.

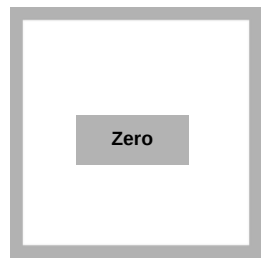
7. Swirl to mix.

8. Start the instrument timer.

A two-minute reaction time will begin.

9. Blank Preparation: When the timer expires, fill a sample cell with 10 mL of the remaining prepared sample.

10. Wipe the blank and insert it into the cell holder.



11. ZERO the instrument.

The display will show:

0.00 mg/L Mo^{6+}

12. Wipe the developed sample and insert it into the cell holder.

READ the results in mg/L Mo^{6+}.

Interferences

Interference studies were conducted by preparing a molybdenum standard solution (2 mg/L Mo^{6+}) containing the potential interfering ion. When the standard solution concentration changed by ± 5% with a given ion concentration, the ion was considered an interference. Interference results are summarized in *Substances that cause a negative interference*, *Substances that cause a positive interference* and *Non-interfering substances*.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagent and require sample pretreatment. Adjust the sample pH to between 3–5 by adding, by drops, an appropriate amount of acid or base such as 1.0 N Sulfuric Acid Standard Solution* or 1.0 N Sodium Hydroxide Standard Solution*. If significant volumes of acid or base are used, a volume correction should be made by dividing the total volume (sample + acid + base) by the original volume and multiplying the test result by this factor.

After a number of samples have been analyzed, the sample cells may exhibit a slight bluish discoloration. Rinse the cells with 1:1 Hydrochloric Acid Solution* to eliminate this build-up.

Table 211 Substances that cause a negative interference

Interfering substance	Interference level
Alum	Greater than 7 mg/L
Aluminum	Greater than 2 mg/L
AMP (Phosphonate)	Greater than 15 mg/L
Bicarbonate	Greater than 5650 mg/L
Bisulfate	Greater than 3300 mg/L
Borate	Greater than 5250 mg/L
Chloride	Greater than 1400 mg/L
Chromium	Greater than 4.5 mg/L[1]
Copper	Greater than 98 mg/L
Diethanoldithiocarbamate	Greater than 32 mg/L
EDTA	Greater than 1500 mg/L
Ethylene Glycol	Greater than 2% (by volume)
Iron	Greater than 200 mg/L
Lignin Sulfonate	Greater than 105 mg/L
Nitrite	Greater than 350 mg/L
Orthophosphate	Greater than 4500 mg/L
Phosphonohydroxyacetic Acid	Greater than 32 mg/L
Phosphonate HEDP	Positive interference of about 10% up to 30 mg/L. As the concentration increases above 30 mg/L, a decrease in the molybdenum concentration reading occurs (negative interference).
Sulfite	Greater than 6500 mg/L

[1] Read the molybdenum concentration immediately after the 2-minute reaction period.

Table 212 Substances that cause a positive interference

Interfering substance	Interference level
Benzotriazole	Greater than 210 mg/L
Carbonate	Greater than 1325 mg/L
Morpholine	Greater than 6 mg/L

* See *Optional reagents and apparatus*.

Table 212 Substances that cause a positive interference (continued)

Interfering substance	Interference level
Phosphonate HEDP	The presence of the phosphonate HEDP at concentrations up to 30 mg/L will increase the apparent molybdenum concentration reading by approximately 10% (positive interference). Multiply the value obtained in step 12 by 0.9 to obtain the actual Mo^{6+} concentration.
Silica	Greater than 600 mg/L

Table 213 Non-interfering substances

Interfering substance	Interference level
Bisulfite	9600 mg/L
Calcium	720 mg/L
Chlorine	7.5 mg/L
Magnesium	8000 mg/L
Manganese	1600 mg/L
Nickel	250 mg/L
PBTC (phosphonate)	500 mg/L
Sulfate	12,800 mg/L
Zinc	400 mg/L

Sample collection, preservation and storage

- Collect samples in glass or plastic bottles.
- Analyze samples immediately.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Molybdenum Standard Solution, 1000-mg/L Mo^{6+}
- Graduated cylinder, 250 mL
- Erlenmeyer flasks (3)
- TenSette Pipet and Pipet tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Select **Options>More>Standard Additions** from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution bottle.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 200-mL portions of fresh sample. Mix thoroughly.
6. Follow the *Ternary Complex Method for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Molybdenum Standard Solution, 10.00-mg/L
- Deionized water
- 50-mL Class A volumetric flask
- 10 mL, Class A volumetric pipet
- Pipet filler

1. Prepare a 2.00-mg/L molybdenum standard solution as follows:
   a. Pipet 10.00 mL of Molybdenum Standard Solution, 10.00-mg/L, into a 50-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use this solution in place of the sample. Follow the *Ternary Complex Method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
315	2.00 mg/L Mo^{6+}	1.94–2.06 mg/L Mo^{6+}	0.02 mg/L Mo^{6+}

Summary of method

The ternary complex method for molybdenum determination is a method in which molybdate molybdenum reacts with an indicator and a sensitizing agent to give a stable blue complex. Test results are measured at 610 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Molybdenum Reagent Set for 20-mL sample (100 tests), includes:	—	—	2449400
(1) Molybdenum 1 Reagent (LR) Molybdate Powder Pillows	1	100/pkg	2352449
(1) Molybdenum 2 Reagent Solution	0.5 mL	50 mL MDB	2352512

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated mixing, 25-mL	1	each	189640
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Molybdenum Standard Solution, 10-mg/L Mo^{6+}	100 mL	1418742
Molybdenum Standard Solution, 1000-mg/L Mo^{6+}	100 mL	1418642
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder graduated, 250 mL	each	108146
Filter Paper, folded, 12.5 cm	100/pkg	189457
Funnel, poly, 65 mm	each	108367
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Flask, Erlenmeyer, 250 mL	each	50546
Pipet, Volumetric Class A, 10 mL	each	1451538
Pipet Filler, Safety Bulb	each	1465100
Hydrochloric Acid Solution 1:1	500 mL	88449
Sodium Hydroxide Standard Solution, 1.0 N	100 mL MDB	104532
Sulfuric Acid Standard Solution, 1.0 N	100 mL MDB	127032

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Edition 7

Nickel

DOC316.53.01064

USEPA[1] Heptoxime Method[2] **Method 8037**

0.02 to 1.8 mg/L Ni **Powder Pillows**

Scope and Application: For Water, Wastewater and Seawater

[1] USEPA accepted for reporting wastewater analyses (digestion required). Procedure is equivalent to Standard Method 3500-Ni D for wastewater

[2] Adapted from *Chemie Analytique*, 36 43 (1954)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 214 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2612602	Fill line faces user
DR 3900	2612602	Fill line faces user
DR 3800, DR 2800, DR 2700	2612602	Fill line faces right

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Make the cotton plug pea-size. A larger plug will restrict the flow; a smaller plug may become dislodged from the delivery tube of the funnel.
Chloroform (D022) solutions are regulated as hazardous waste. Do not pour these materials down the drain. Water saturated with chloroform, chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for proper disposal. Refer to a current MSDS for safe handling and disposal instructions.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the DR 3900, DR 3800, DR 2800 and DR 2700 cell compartment with the protective cover during measurements.

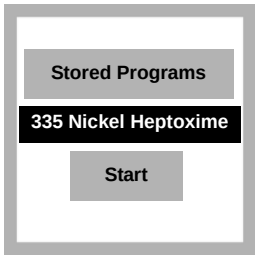
Collect the following items :

Description	Quantity
Chloroform, ACS	30 mL
Nickel 1 Reagent Powder Pillow	1
Nickel 2 Reagent Powder Pillow	1

Collect the following items (continued):

Description	Quantity
Clippers for Opening Pillows	1
Cotton Balls	varies
Cylinder, graduated. 10-mL	1
Cylinder, graduated, 500-mL	1
Funnel, separatory with stand and stopper	1
Sample Cells (see *Instrument-specific information*)	2
See *Consumables and replacement items* for reorder information.	

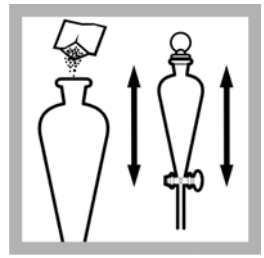
Heptoxime method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

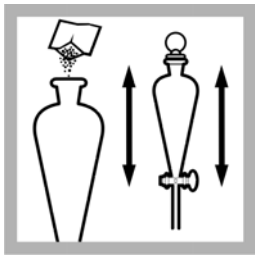
2. Measure 300 mL of sample in a 500-mL graduated cylinder. Pour into a 500-mL separatory funnel.



3. Add the contents of one Nickel 1 Reagent Powder Pillow to the funnel. Stopper and invert to mix.

4. Start the instrument timer.

A five-minute reaction time will begin.

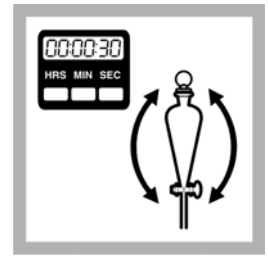


5. When the timer expires, add the contents of one Nickel 2 Reagent Powder Pillow to the funnel. Stopper and invert to mix.

6. Start the instrument timer.

A second five-minute reaction time will begin.

7. When the timer expires, add 10 mL of chloroform. Insert the stopper and invert gently. With the funnel inverted and the tip pointed away from people, open the stopcock to vent.



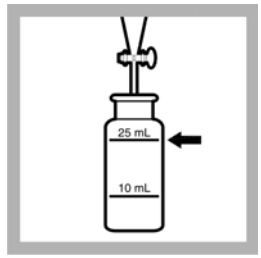
8. Close the stopcock and invert for 30 seconds.

Heptoxime method for powder pillows (continued)

9. Start the instrument timer.

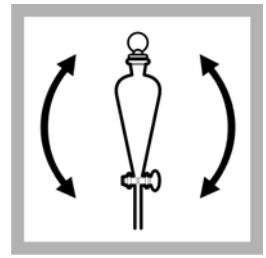
A third five-minute reaction period will begin.

Invert the funnel several times over the five minute period.

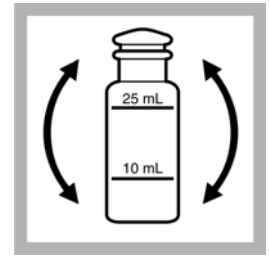


10. Prepared Sample: When the timer expires, wait for the layers to separate. Insert a pea-sized cotton plug into the delivery tube of the funnel. Remove the stopper and drain the chloroform layer (bottom layer) into a sample cell.

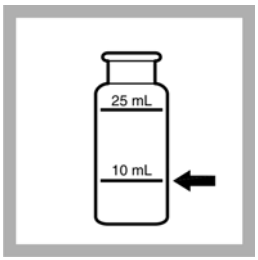
Insert the stopper into the funnel.



11. Repeat steps 7 through 10 two additional times with 10-mL volumes of chloroform. The five-minute reaction period is not necessary.



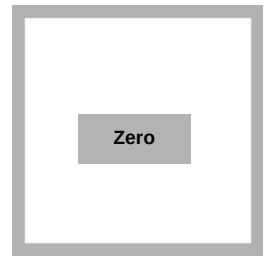
12. Cap the sample cell and invert to mix the extracts. The final volume will be about 25 mL due to the slight solubility of chloroform in water.



13. Blank Preparation: Fill a second sample cell with 10 mL of chloroform.

Cap the cell.

14. Wipe the blank and insert it into the cell holder.



15. ZERO the instrument.

The display will show:

0.00 mg/L Ni

16. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L Ni.

Interferences

Cobalt, copper and iron interferences can be overcome by adding additional Nickel 1 Reagent Powder Pillows in step 3 of the *Heptoxime method for powder pillows*. The tolerance limits of these interferences are shown in the *Interfering substances* table.

A preliminary acid digestion is required to determine any suspended or precipitated nickel and to eliminate interference by organic matter. To eliminate this interference or to determine total recoverable nickel perform the USEPA approved digestion.

Table 215 Interfering substances

Pillows of Nickel 1 Reagent	Tolerance Limit (mg/L)		
	Cobalt	Copper	Iron
1	1	10	20
2	7	16	65
3	13	22	110
4	18	28	155
5	25	35	200

Sample collection, preservation and storage

- Collect samples in acid-washed plastic bottles.
- Adjust the sample pH to 2 or less with Nitric Acid*, about 5 mL per liter. Preserved samples can be stored up to six months at room temperature.
- Before analysis, adjust the sample pH to between 3–8 with 5.0 N Sodium Hydroxide Standard Solution*. Do not exceed pH 8 as this may cause some loss of nickel as a precipitate.
- Correct the test results for volume additions.

Accuracy check

Standard additions method (sample spike)
Prepare a 300 mg/L nickel standard by pipetting 15 mL of 1000 mg/L Nickel standard into a 50 ml volumetric flask. Dilute to volume and mix well.

Required for accuracy check:

- Nickel Standard Solution, 1000-mg/L Ni
- Volumetric Pipet, 15 mL
- Volumetric Flask, 50 mL
- TenSette Pipet and tips
- Pipet Filler

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **Options>More>Standard Additions** from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL and 0.6 mL of 300 mg/L standard to three 300-mL portions of fresh sample.
5. Follow the *Heptoxime method for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
6. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

* See *Optional reagents and apparatus*.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Nickel Standard Solution, 1000-mg/L
- Deionized water
- Volumetric flasks, 500 mL and 1000 mL
- Volumetric pipets, 10 mL and 50 mL
- Pipet filler

1. Prepare a 10.0 mg/L nickel working solution as follows:
   a. Pipet 10.0 mL of a Nickel Standard Solution, 1000-mg/L, into a 1000-mL (1 liter) volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Prepare a 1.0-mg/L nickel standard solution by diluting 50.0 mL of the 10-mg/L working standard solution to 500 mL in a volumetric flask.
3. Use the standard solution in place of the sample. Follow the *Heptoxime method for powder pillows* test procedure.
4. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
335	1.00 mg/L Ni	0.93–1.07 mg/L Ni	0.02 mg/L Ni

Summary of method

Nickel ion reacts with heptoxime to form a yellow-colored complex which is then extracted into chloroform to concentrate the color and enable a more sensitive determination. Chelating agents are added to the sample to overcome the interferences caused by cobalt, copper and iron. Readings are taken at 430 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Nickel Reagent Set (50 Tests), includes:	—	—	2243500
(3) Chloroform, ACS	30 mL	500 mL	1445849
(2) Nickel 1 Reagent Powder Pillows	1	25/pkg	212368
(2) Nickel 2 Reagent Powder Pillows	1	25/pkg	212468

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers	1	each	96800
Cotton Balls, absorbent	1	100/pkg	257201
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 500-mL	1	each	50849
Funnel, separatory, 500-mL	1	each	52049
Ring, support, 4-inch	1	each	58001
Sample Cell, 1-inch square, w/stopper, matched pair	2	2/pkg	2612602
Stand, support, 5 x 8-inch base	1	each	56300

Recommended standards

Description	Unit	Catalog number
Nickel Standard Solution, 1000-mg/L Ni (NIST)	100 mL	1417642
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25 mL	each	189640
Pipet, volumetric, Class A, 15 mL	each	1451539
Pipet, Volumetric, Class A, 50 mL	each	1451541
Flask, Volumetric, Class A, 500 mL	each	1457449
Flask, Volumetric, Class A, 1000 mL	each	1457453
Flask, Volumetric, Class A, 50 mL	each	1457441
Pipet Filler, Safety Bulb	each	1465100
Nitric Acid 1:1	500 mL	254049
Sodium Hydroxide Standard Solution, 5.0 N	100 mL	245053
Pipet, volumetric, 10 mL	each	1451538

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Edition 7

Nickel

DOC316.53.01063

1-(2 Pyridylazo)-2-Napthol (PAN) Method[1] **Method 8150**

0.006 to 1.000 mg/L Ni **Powder Pillows**

Scope and Application: For water and wastewater; digestion is required for determining total nickel

[1] Adapted from Watanabe, H., *Talanta*, 21 295 (1974)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 216 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

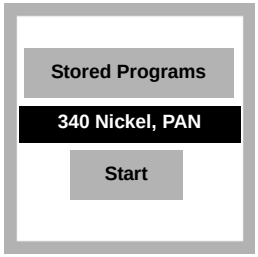
Cobalt concentration can be determined with the same sample by using Program Number 110.
If the sample is less than 10 °C (50 °F), warm to room temperature before analysis. Adjust the pH of acidified stored samples.

Collect the following items:

Description	Quantity
EDTA Powder Pillow	2
Phthalate-Phosphate Reagent Powder Pillow	2
PAN Indicator Solution, 0.3%	1 mL
Deionized Water	25 mL
Sample Cells (see *Instrument-specific information*)	2
Stoppers	2

See *Consumables and replacement items* for reorder information.

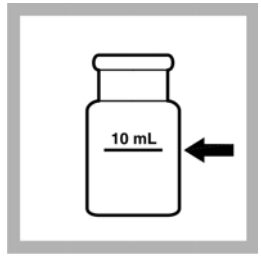
PAN method for powder pillows



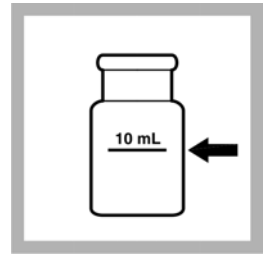
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Prepared Sample: Fill a sample cell to the 10-mL mark with sample.



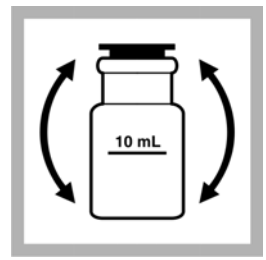
3. Blank Preparation: Fill a second sample cell to the 10-mL mark with deionized water.

4. Add the contents of one Phthalate-Phosphate Reagent Powder Pillow to each cell.

5. Stopper the cells. Immediately shake to dissolve.

If the sample contains iron, make sure that all the powder is dissolved before proceeding to step 6.

6. Using the plastic dropper provided, add 0.5 mL of 0.3% PAN Indicator Solution to each cell.



7. Insert stoppers into the cells. Invert several times to mix.

8. Start the instrument timer.

A 15-minute reaction period will begin.

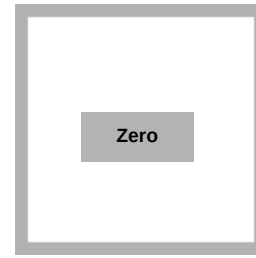
During color development, the sample solution color may vary from yellowish-orange to dark red, depending on the chemical makeup of the sample. The blank should be yellow.

PAN method for powder pillows (continued)

9. When the timer expires, add the contents of one EDTA Reagent Powder Pillow to each cell.

10. Stopper the cells and shake to dissolve.

11. Wipe the blank and insert it into the cell holder



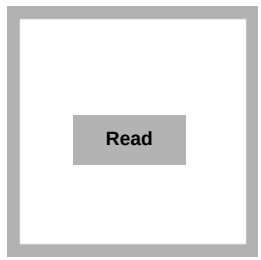
12. **ZERO** the instrument.

The display will show:

0.00 mg/L Ni

The instrument will zero at 560 and 620 nm.

13. Wipe the sample cell and insert it into the cell holder.



14. **READ** the results in mg/L Ni and Co.

Interferences

Table 217 Interfering substances

Interfering substance	Interference level
Al^{3+}	32 mg/L
Ca^{2+}	1000 mg/L as ($CaCO_3$)
Cd^{2+}	20 mg/L
Cl^-	8000 mg/L
Chelating agents (e.g., EDTA)	Interfere at all levels. Use either the Digesdahl or vigorous digestion to eliminate this interference.
Cr^{3+}	20 mg/L
Cr^{6+}	40 mg/L
Cu^{2+}	15 mg/L
F^-	20 mg/L
Fe^{3+}	10 mg/L

Table 217 Interfering substances (continued)

Interfering substance	Interference level
Fe^{2+}	Interferes directly and must not be present.
K^{+}	500 mg/L
Mg^{2+}	400 mg/L
Mn^{2+}	25 mg/L
Mo^{6+}	60 mg/L
Na^{+}	5000 mg/L
Pb^{2+}	20 mg/L
Zn^{2+}	30 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

- Collect samples in acid-washed plastic bottles.
- Adjust the sample pH to 2 or less with Nitric Acid*, about 5 mL per liter. Preserved samples can be stored up to six months at room temperature.
- Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution*. Do not exceed pH 8 as this may cause some loss of nickel as a precipitate.
- If the sample is less than 10 °C, warm it to room temperature.
- Correct test results for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Nickel Standard solution, 1000-mg/L Ni
- 25 mL mixing cylinders (3)
- 5-mL Volumetric Pipet, Class A
- TenSette Pipet and Pipet Tips
- 100 mL Volumetric Flask
- Pipet Filler
- Deionized Water

1. Prepare a 50 mg/L Nickel standard by pipetting 5.00 mL of 1000 mg/L Ni standard solution into a 100 mL volumetric flask. Dilute the solution to the required volume and mix well.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select **Options>More>Standard Additions** from the instrument menu.
4. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.

* See *Optional reagents and apparatus*.

5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample. Mix well.
6. Transfer 10-mL of each solution into sample cells. Follow the *PAN method for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Nickel Standard Solution, 1000-mg/L as Ni
- Deionized water
- 1-L volumetric flask, Class A
- 100-mL Volumetric flask
- Volumetric pipets, 5 mL and 10 mL
- Pipet filler

1. Prepare a 5.00 mg/L nickel stock solution as follows:
   a. Pipet 5.00 mL of Nickel Standard Solution, 1000-mg/L as Ni, into a 1000-mL (1 liter) volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Prepare a 0.5 mg/L nickel working solution as follows:
   a. 10.0 mL of the 5.00-mg/L nickel stock solution into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
3. Use the working solution in place of the sample. Follow the *PAN method for powder pillows* test procedure.
4. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
340	0.500 mg/L Ni	0.492–0.508 mg.L Ni	0.006 mg/L Ni

Summary of method

After buffering the sample and masking any Fe^{3+} with pyrophosphate, the nickel is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt. The instrument automatically adjusts for cobalt interference by measuring the absorbance

of the sample at both 560 nm and 620 nm. This method is unique because both nickel and cobalt can be determined on the same sample when using a spectrophotometer.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Nickel Reagent Set (100 Tests), includes:	—	—	2651600
(2) EDTA Reagent Powder Pillows	2	100/pkg	700599
(2) Phthalate-Phosphate Reagent Powder Pillows	2	100/pkg	2615199
(1) PAN Indicator Solution, 0.3%	1 mL	100 mL MDB	2150232
Water, deionized	25 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stoppers	2	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Nickel Standard Solution, 1000-mg/L Ni (NIST)	100 mL	1417642

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25 mL	each	189640
Flask, Volumetric, Class A, 100 mL	each	1457442
Pipet, Volumetric, Class A, 5 mL	each	1451537
Pipet Filler, Safety Bulb	each	1465100
Pipet, volumetric, Class A, 10 mL	each	1451538
Flask, volumetric, Class A, 1000 mL	each	1457453
Water, deionized	4 L	27256
Nitric Acid 1:1	500 mL	254049
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032

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Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 218 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

For DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield before performing this test.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water (nitrate-free) in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.

Collect the following items:

Description	Quantity
Light Shield (see *Instrument-specific information*)	1
Test ‘N Tube™ NitraVer® X Reagent Set	1
Funnel, micro, poly	1
Pipet, TenSette®, 0.01 to 1.0 mL, plus tips	1
Test Tube Rack	1

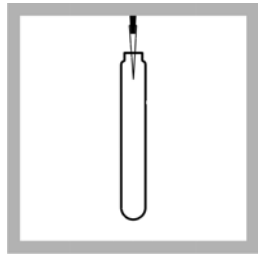
See *Consumables and replacement items* for reorder information.

Chromotropic Acid method for TNT

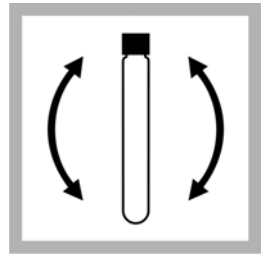
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

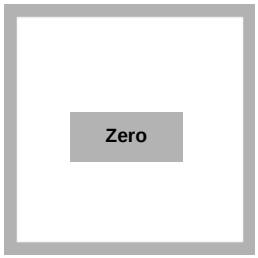


2. Blank Preparation: Remove the cap from a NitraVer X Reagent A Test 'N Tube vial and add 1.00 mL of sample.



3. Cap the tube and invert ten times to mix.

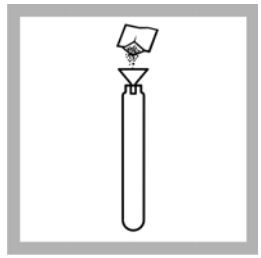
4. Wipe the blank and insert it into the 16-mm cell holder.



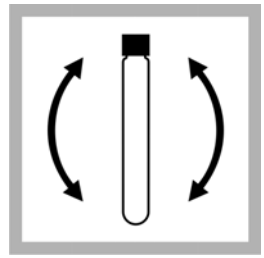
5. ZERO the instrument.

The display will show:

0.0 mg/L NO^{3-}–N



6. Prepared Sample: Remove the vial from the instrument. Using a funnel, add the contents of one NitraVer X Reagent B Powder Pillow to the vial.



7. Cap and invert ten times to mix.

Some solid matter will not dissolve.

8. Start the instrument timer.

A five-minute reaction time will begin. Do not invert the vial again.

A yellow color will develop if nitrate is present.

9. Within five minutes after the timer expires, wipe the prepared sample and insert it into the cell holder.

10. READ the results in mg/L NO_3^-–N.

To display other chemical forms, refer to the instrument user manual.

Interferences

Table 219 Interfering substances

Interfering substance	Interference level
Barium	A negative interference at concentrations greater than 1 mg/L.
Chloride	Does not interfere below 1000 mg/L.
Nitrite	A positive interference at concentrations greater than 12 mg/L. (Remove nitrite interference up to 100 mg/L by adding 400 mg (one full 0.5 g Hach measuring spoon) of Urea to 10 mL of sample. Swirl to dissolve. Proceed with the nitrate test as usual.)
Copper	Positive interference at all levels.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Store the samples at 4 °C (39 °F) or lower if the sample will be analyzed within 24 to 48 hours. For longer storage periods (up to 14 days), adjust sample pH to 2 or less with concentrated Sulfuric Acid, ACS* (about 2 mL per liter). Sample refrigeration is still required.
- Before testing, warm the stored sample to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution*. Do not use mercury compounds as preservatives.
- Correct the test result for volume additions:

$$\text{Corrected result} = \frac{(\text{acid volume} + \text{base volume} + \text{sample volume})}{\text{original sample volume}} \times \text{test result}$$

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- High Range Nitrate Nitrogen Voluette® Ampule Standard, 500 mg/L NO_3^-–N
- Ampule breaker
- TenSette Pipet and Pipet Tips
- 25 mL mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **Options>More>Standard Additions** from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three mixing cylinders with 25-mL portions of fresh sample.
6. Add 1 mL of the 0.1 mL spiked sample to a TNT vial and follow the *Chromotropic Acid method for TNT* test procedure. Repeat for each spiked sample. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

* See *Optional reagents and apparatus*.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 10.0-mg/L Nitrate Nitrogen Standard Solution

1. Use the 10.0-mg/L Nitrate Nitrogen Standard Solution solution in place of the sample. Follow the *Chromotropic Acid method for TNT* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
344	10.0 mg/L NO_3^-–N	9.5–10.5 mg/L NO_3^-–N	0.2 mg/L NO_3^-–N

Summary of method

Nitrate in the sample reacts with chromotropic acid under strongly acidic conditions to yield a yellow product with a maximum absorbance at 410 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Test 'N Tube™ NitraVer® X Nitrate Reagent Set	1	50/pkg	2605345

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
Funnel, micro, poly	1	each	2584335
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	varies	50/pkg	2185696
Test Tube Rack	1	each	1864100

Recommended standards

Description	Unit	Catalog number
Nitrate Nitrogen Standard Solution, 10-mg/L: $N0_3$–N	500 mL	30749
Nitrate Nitrogen Standard Solution, Voluette® Ampule, 500-mg/L $N0_3$–N	16/pkg	1426010
Wastewater Influent Inorganics Standard for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule breaker	each	2196800
Cylinder, mixing, 25 mL	each	2088640
Pipet Tips, for TenSette Pipet 1970001	each	1000/pkg
Sodium Hydroxide, 5.0 N	50 mL SCDB	245026
Sulfuric Acid ACS, Concentrated	500 mL	97949
Spoon, measuring, 0.5 g	each	90700
Urea, ACS grade	100 g	1123726

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Edition 7

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 220 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

A deposit of unoxidized metal will remain at the bottom of the cell after the NitraVer® 5 dissolves. The deposit will not affect results.

This method is technique-sensitive. Shaking time and technique influence color development. For most accurate results, make successive tests on a 10-mg/L Nitrate Nitrogen Standard solution. Adjust shaking time and technique to obtain the correct result. Use this technique for all the samples.

Rinse the sample cell immediately after use to remove all cadmium particles. Prepared samples will contain cadmium and must be disposed of according to federal, state and local hazardous waste regulations. Refer to the current MSDS for safe handling and disposal instructions.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
NitraVer® 5 Nitrate Reagent Powder Pillow	1
Sample Cells, 1-inch, 10-mL, with stopper	2
AccuVac Test:	
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample Cell, for blank	1

See *Consumables and replacement items* for reorder information.

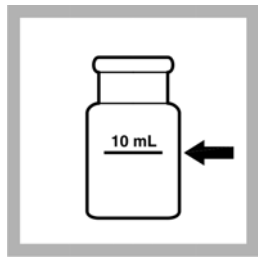
Cadmium Reduction Method for powder pillows

CAUTION
Hazardous waste exposure. Prepared samples contain cadmium. Refer to the current MSDS for safe handling and disposal instructions. Follow all federal, state and local hazardous waste disposal regulations.

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a sample cell with 10 mL of sample.

3. Prepared Sample: Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow. Stopper.

4. Start the instrument timer. A one-minute reaction time will begin.

Cadmium Reduction Method for powder pillows (continued)

CAUTION

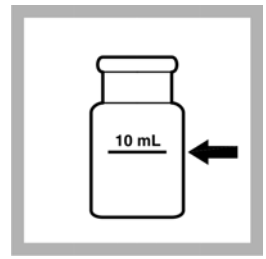
Hazardous waste exposure. Prepared samples contain cadmium. Refer to the current MSDS for safe handling and disposal instructions. Follow all federal, state and local hazardous waste disposal regulations.

5. Shake the cell vigorously until the timer expires.

Note: *Some solid materials may not dissolve.*

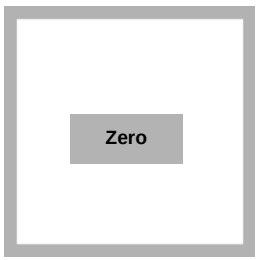
6. When the timer expires, start the timer again. A five-minute reaction period will begin.

An amber color will develop if nitrate is present.



7. Blank Preparation: When the timer expires, fill a second sample cell with 10 mL of sample.

8. Wipe the blank and insert it into the cell holder.

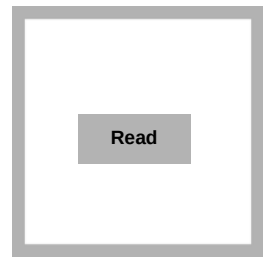


9. **ZERO** the instrument.

The display will show:

0.0 mg/L NO_3^-–N

10. Within one minute after the timer expires, wipe the prepared sample and insert it into the cell holder.



11. **READ** the results in mg/L NO_3^-–N.

To display other chemical forms, refer to the user manual.

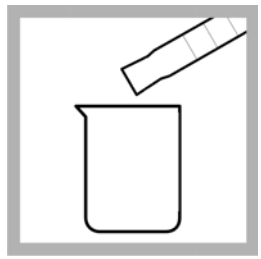
Cadmium Reduction Method for AccuVac® Ampuls

CAUTION
Hazardous waste exposure. Prepared samples contain cadmium. Refer to the current MSDS for safe handling and disposal instructions. Follow all federal, state and local hazardous waste disposal regulations.

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

3. Tap the bottom of a NitraVer 5 Nitrate AccuVac® Ampul on a hard surface to dislodge powder. Fill the Ampul with sample. Keep the tip immersed while the Ampul fills completely. Insert a cap over the Ampul tip.

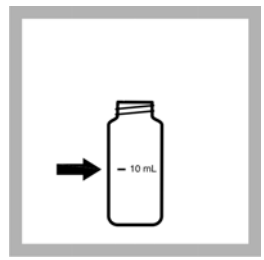
4. Start the instrument timer.

A one-minute reaction period will begin.

5. Invert the Ampul 48–52 times as the timer counts down.

6. When the timer expires, start the timer again. A five-minute reaction period will begin. Do not agitate or disturb the sample during this time.

An amber color will develop if nitrate is present.



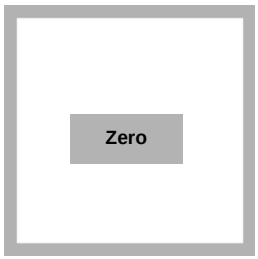
7. Blank Preparation: When the timer expires, fill a round sample cell with 10 mL of sample.

8. Wipe the blank and insert it into the cell holder.

Cadmium Reduction Method for AccuVac® Ampuls (continued)

CAUTION

Hazardous waste exposure. Prepared samples contain cadmium. Refer to the current MSDS for safe handling and disposal instructions. Follow all federal, state and local hazardous waste disposal regulations.

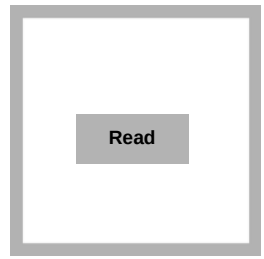


9. **ZERO** the instrument.

The display will show:

0.0 mg/L NO_3^-–N

10. Within one minute after the timer expires, wipe the Ampul and insert into the cell holder.



11. **READ** the results in mg/L NO_3^-–N.

Interferences

Table 221 Interfering substances

Interfering substance	Interference level
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater), but a calibration must be done using standards spiked to the same chloride concentration. Refer to *Seawater calibration*.
Ferric iron	Interferes at all levels
Nitrite	Interferes at all levels Compensate for nitrite interference as follows: Before performing step 3, add 30-g/L Bromine Water[1] dropwise to the sample until a yellow color remains. Add one drop of 30-g/L Phenol Solution[1] to destroy the color. Proceed with step 3. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

[1] See *Optional reagents and apparatus*.

Seawater calibration

Chloride concentrations above 100 mg/L will cause low results. To perform this test in water with high interference level, calibrate the water using standards spiked to the same chloride concentrations as the required samples. To prepare calibration standards containing 5.0, 10.0, 20.0 and 30.0 mg/L nitrate as NO_3^-–N:

1. Prepare a 1 L volume of chloride water that matches the concentration of the samples, using the following equation:

   c. Add necessary Chloride concentration (g/L) x (1.6485) = g of ACS grade NaCl to 1 L of deionized water. 18.8 g/L is a typical seawater chloride concentration.

   d. Mix this solution thoroughly to get a homogeneous solution. Use this water as the dilution water instead of the deionized water when preparing the nitrate standards.

2. Use Class A glassware or a Tensette Pipet to pipet 0.5, 1.0, 2.0, and 3.0 mL of the 1000 mg/L Nitrogen-Nitrate as NO_3^-–N (NIST) Standard Solution (Catalog Number 1279249) into four different 100 mL Class A volumetric flasks.

3. Dilute to the mark with the prepared chloride water. Mix thoroughly.

4. Use the prepared chloride water for the 0-mg/L nitrate as NO_3^-–N standard.

Sample collection, preservation and storage

- More reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (H_2SO_4)* per liter and store at 4 °C. The results are reported as total nitrate plus nitrite.
- Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution*. Do not use mercury compounds as preservatives.
- Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy check

Standard additions method for powder pillows (sample spike)
Required for accuracy check:

- Nitrate Nitrogen Standard solution, 1000-mg/L NO_3^-–N
- Mixing cylinders
- 25-mL Volumetric pipet
- TenSette Pipet and Pipet Tips
- Pipet filler
- 100 mL Volumetric Flask

1. Prepare a 250-mL nitrate nitrogen standard solution by pipetting 25 mL of a 1000 mg/L Nitrate Nitrogen standard solution into a 100 mL volumetric flask. Dilute the solution to the required volume with deionized water and mix thoroughly.

2. After reading test results, leave the sample cell (unspiked sample) in the instrument.

3. Select **Options>More>Standard Additions** from the instrument menu.

* See *Optional reagents and apparatus*.

4. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
6. Follow the *Cadmium Reduction Method for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)

1. Fill three mixing cylinders each with 50-mL of sample and spike with 0.4 mL, 0.8 mL and 1.2 mL of 250-mg/L standard.
2. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
3. Analyze each standard addition sample as described in the *Cadmium Reduction Method for AccuVac® Ampuls*.
4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 10.0-mg/L Nitrate Nitrogen Standard Solution

1. Use the 10.0-mg/L Nitrate Nitrogen Standard Solution in place of the sample. Follow the *Cadmium Reduction Method for powder pillows* and *Cadmium Reduction Method for AccuVac® Ampuls* test procedures.
2. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change	
			Point of curve	Concentration
355	10 mg/L NO_3^-–N	9.3–10.7 mg/L NO_3^-–N	0 ppm	0.3 mg/L NO_3^-–N
			10 ppm	0.5 mg/L NO_3^-–N
			30 ppm	0.8 mg/L NO_3^-–N
361	10 mg/L NO_3^-–N	9.3–10.7 mg/L NO_3^-–N	0 ppm	0.5 mg/L NO_3^-–N
			10 ppm	0.6 mg/L NO_3^-–N
			30 ppm	0.8 mg/L NO_3^-–N

Summary of method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. Test results are measured at 500 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitraVer® 5 Nitrate Reagent Powder Pillows (for 10-mL sample)	1	100/pkg	2106169
OR			
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1	25/pkg	2511025

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stopper, Neoprene, solid, size #1	2	12/pkg	1480801
or			
Stopper	2	6/pkg	173106

Required apparatus (AccuVac)

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606

Recommended standards

Description	Unit	Catalog number
Nitrate Nitrogen Standard Solution, 10.0-mg/L NO_3^-–N	500 mL	30749
Nitrate Nitrogen Standard Solution 1000 mg/L NO_3^-–N	500 mL	1279249
Wastewater Influent Standard, Mixed Parameter, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water, 30 g/L	29 mL	221120
Cylinder, mixing, 50 mL	each	2088641
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet, Volumetric, Class A, 0.5 mL	each	1451534
Pipet, Volumetric, Class A, 1.0 mL	each	1451535
Pipet, Volumetric, Class A, 2.0 mL	each	1451536
Pipet, Volumetric, Class A, 3.0 mL	each	1451503
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	2185696

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Pipet Tips, for TenSette Pipet 19700-01	1000/pkg	2185628
Phenol Solution, 30 g/L	29 mL	211220
Pipet, Volumetric, Class A, 25 mL	each	1451540
Pipet Filler, Safety Bulb	each	1465100
AccuVac Snapper	each	2405200
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	245026
Sulfuric Acid, concentrated, ACS	500 mL	97949
Flask, volumetric, Class A, 100 mL	each	1457442

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Edition 7

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 222 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
A deposit of unoxidized metal will remain at the bottom of the cell after the NitraVer® 6 dissolves. The deposit will not affect results.
Shaking time and technique influence color development. Analyze a standard solution several times and adjust the shaking time to obtain the correct result. Use this technique for analyzing samples. See the *Standard solution method*.
Rinse the sample cell and mixing cylinder immediately after use to remove all cadmium particles.
Properly dispose of the used sample. Prepared samples contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. Refer to the current MSDS for safe handling and disposal information.

Collect the following items:

Description	Quantity
NitraVer® 6 Nitrate Reagent powder pillow	1
NitriVer® 3 Nitrite Reagent powder pillow	1
Cylinder, graduated, mixing, 25-mL	1
Sample Cells (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

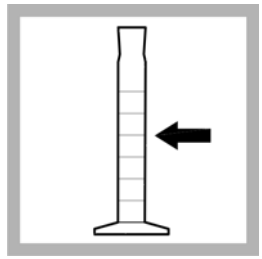
Cadmium Reduction Method for powder pillows

CAUTION
Hazardous waste exposure. Prepared samples contain cadmium. Refer to the current MSDS for safe handling and disposal instructions. Follow all federal, state and local hazardous waste disposal regulations.

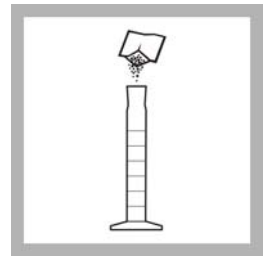
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



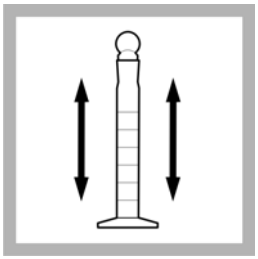
2. Fill a 25-mL graduated mixing cylinder with 15 mL of sample.



3. Add the contents of one NitraVer 6 Reagent Powder Pillow to the cylinder. Insert the stopper.

4. Start the instrument timer.

A three-minute reaction time will begin.

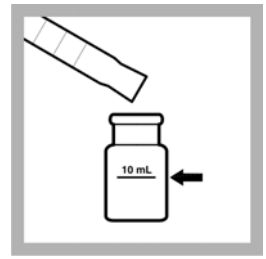


5. Shake the cylinder vigorously during the three-minute timer.

Note: *Some solid materials may not dissolve.*

6. When the timer expires, start the instrument timer again.

A two-minute reaction period will begin.



7. When the timer expires, carefully pour 10 mL of the sample into a clean sample cell. Do not transfer any cadmium particles to the sample cell.

8. Prepared Sample: Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow to the sample cell.

Cadmium Reduction Method for powder pillows (continued)

CAUTION

Hazardous waste exposure. Prepared samples contain cadmium. Refer to the current MSDS for safe handling and disposal instructions. Follow all federal, state and local hazardous waste disposal regulations.

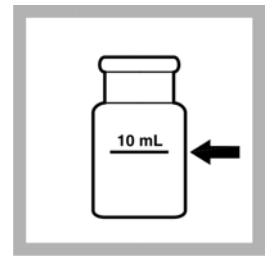
9. Start the instrument timer. A 30-second reaction time will begin.

10. Cap and shake the sample cell gently during the 30-second timer.

A pink color will develop if nitrate is present.

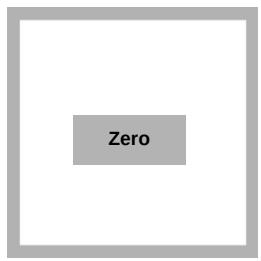
11. Start the instrument timer.

A 15-minute reaction period will begin.



12. Blank Preparation: When the timer expires, fill a second square sample cell with 10 mL of original sample.

13. Wipe and insert the blank into the cell holder.

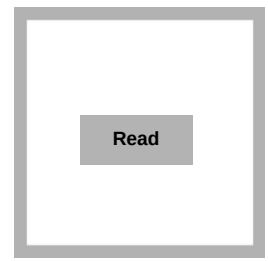


14. ZERO the instrument.

The display will show:

0.00 mg/L NO_3^-–N

15. Wipe and insert the prepared sample into the cell holder.



16. READ the results in mg/L NO_3^-–N.

Interferences

Table 223 Interfering substances

Interfering substance	Interference level
Calcium	100 mg/L
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration. (Refer to Sea Water Calibration.)
Ferric iron	All levels
Nitrite	All levels: This method measures both the nitrate and nitrite in the sample. If nitrite is present, the nitrite nitrogen test (Program #371) should be done on the sample. Pretreat the nitrate nitrogen sample with the following pretreatment. Then subtract the amount of nitrite found from the results of the LR nitrate nitrogen test. **1.** Add 30-g/L Bromine Water[1] dropwise to the sample in step 3 until a yellow color remains. Mix after each drop. **2.** Add one drop of 30-g/L Phenol Solution[1] to destroy the color. **3.** Proceed with the *Cadmium Reduction Method for powder pillows*.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interferes at all levels

[1] See *Optional reagents and apparatus*.

Seawater calibration

Chloride concentrations above 100 mg/L will cause low results. To perform this test in water with high-interference level, calibrate the water using standards spiked to the same chloride concentrations as the required samples. To prepare calibration standards containing 0.06, 0.1, 0.3 and 0.4 mg/L nitrate as NO_3–N:

1. Prepare a 1 L volume of chloride water that matches the concentration of the samples, using the following equation:

   e. Add necessary Chloride concentration (g/L) x (1.6485) = g of ACS grade NaCl to 1 L of deionized water. (

   Note: *18.8 g/L is a typical seawater chloride concentration.*

   f. Mix this solution thoroughly to make sure that it is a homogeneous solution. Use this water as the dilution water instead of the deionized water when preparing the nitrate standards.

2. Use Class A glassware or a Tensette Pipet to pipet 0.6, 1, 3 and 4 mL of the 10 mg/L Nitrogen-Nitrate as NO3-N (NIST) Standard Solution (Catalog Number 30749) into four different 100 mL Class A volumetric flasks.

3. Dilute to the mark with the prepared chloride water. Mix thoroughly.

4. Use the prepared chloride water for the 0-mg/L nitrate as NO_3–N standard.

Sample collection, preservation and storage

- More reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 48 hours at 4 °C. The results will be in total nitrate and plus nitrate.
- To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid* per liter and store at 4 °C.

- Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution*. Do not use mercury compounds as preservatives.
- Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Nitrate Nitrogen Standard, Solution, 100-mg/L NO_3^-–N
- 25 mL mixing cylinders (3)
- TenSette Pipet 1–10 mL and tips
- 50-mL Volumetric Flask
- Pipet filler

1. Prepare a 12-mL nitrate nitrogen standard solution by pipetting 6.0 mL of a 100 mg/L Nitrate Nitrogen standard solution into a 50 mL volumetric flask. Dilute the solution to required volume with deionized water and mix thoroughly.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select **Options>More>Standard Additions** from the instrument menu.
4. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
5. Use the TenSette Pipet to prepare spiked samples in three mixing cylinders: add 0.1 mL, 0.2 mL and 0.3 mL of 12 mg/L standard to three 15-mL portions of fresh sample.
6. Follow the *Cadmium Reduction Method for powder pillows* test procedure for each of the spiked samples using the powder pillows, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Nitrate Nitrogen Standard Solution, 10-mg/L
- Deionized water
- Volumetric flask, 100 mL
- Volumetric pipet, 4 mL

1. Prepare a 0.40-mg/L NO_3^-–N standard solution as follows:
   a. Pipet 4.00 mL of Nitrate Nitrogen Standard Solution, 10 mg/L, into a 100 mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.

* See *Optional reagents and apparatus*.

2. Use this solution in place of the sample. Follow the *Cadmium Reduction Method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
351	0.40 mg/L NO^{3-}–N	0.35–0.45 mg/L NO^{3-}–N	0.003 mg/L NO^{3-}–N

Summary of method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with chromotropic acid to form a pink-colored product. Test results are measured at 507 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Low Range Nitrate Reagent Set (100 tests), includes:	—	—	2429800
NitraVer® 6 Nitrate Reagent Powder Pillows	1	100/pkg	2107249
NitriVer® 3 Nitrite Reagent Powder Pillows	1	100/pkg	2107169

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated mixing, 25-mL	1	each	2088640
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stopper	1	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Flask, volumetric, Class A, 100-mL	each	1457442
Nitrate Nitrogen Standard Solution, 10.0 mg/L NO_3^-–N	500 mL	30749
Nitrate Nitrogen Standard Solution, 100 mg/L NO_3^-–N	500 mL	194749
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water, 30 g/L	29 mL	221120
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, Class A, 4.00 mL	each	1451504
Flask, Volumetric, 50 mL	each	1457441
Pipet filler, safety bulb	each	1465100
Phenol Solution, 30-g/L	29 mL	211220
Sodium Hydroxide Standard Solution, 5.0 N	1 L	245053
Sulfuric Acid, concentrated	500 mL	97949
Sodium chloride, ACS	454 g	18201H
Pipet, TenSette 1–10 mL	each	1970010
Pipet Tips for TenSette Pipet 1970010	50/pkg	2199796

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Edition 7

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 224 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.

A deposit of unoxidized metal will remain at the bottom of the cell after the NitraVer® 5 dissolves. The deposit will not affect results.

This method is technique-sensitive. Shaking time and technique influence color development. For most accurate results, make successive tests on a 10.0-mg/L Nitrate Nitrogen Standard solution. Adjust shaking times to obtain the correct result.

Rinse the sample cell immediately after use to remove all cadmium particles. Retain the used sample for proper hazardous waste disposal for cadmium.

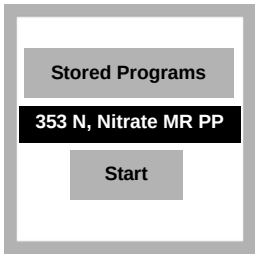
Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. Refer to the current MSDS for safe handling and disposal instructions.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
NitraVer® 5 Nitrate Reagent Powder Pillow	1
Sample Cells (see *Instrument-specific information*)	2
Stopper, Neoprene, #1, solid	2
AccuVac Test:	
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample Cell for blank (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

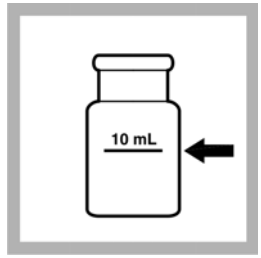
Cadmium reduction method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a sample cell with 10 mL of sample.

3. Prepared Sample: Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow. Insert a stopper into the cell.

4. Start the instrument timer.

A one-minute reaction period will begin.

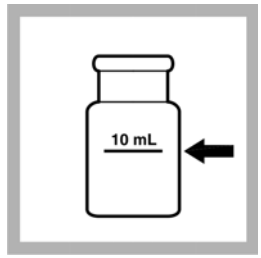
Shake the cell vigorously until the timer expires.
Note: *Some solid material will not dissolve.*

Cadmium reduction method for powder pillows (continued)

5. When the timer expires, start the timer again.

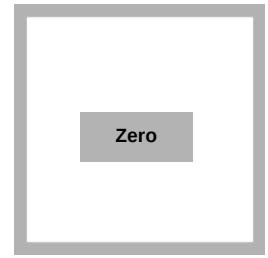
A five-minute reaction period will begin.

An amber color will develop if nitrate is present.



6. Blank Preparation: When the timer expires, fill a second sample cell with 10 mL of sample.

7. Wipe and insert the blank into the cell holder.

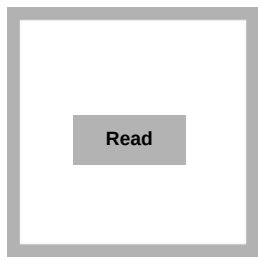


8. **ZERO** the instrument.

The display will show:

0.0 mg/L NO_3^-–N

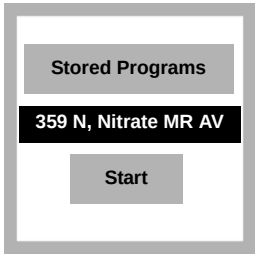
9. Within two minutes after the timer expires, wipe and insert the prepared sample into the cell holder.



10. **READ** the results in mg/L NO_3^-–N.

Refer to the user manual to display other chemical forms.

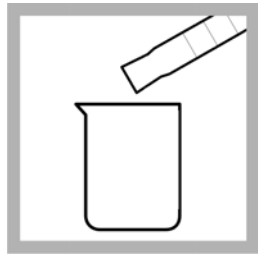
Cadmium reduction method for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

3. Fill a NitraVer 5 Nitrate AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely. Place a stopper over the Ampul tip.

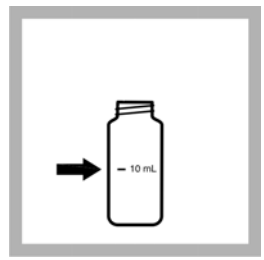
4. Start the instrument timer.

A one-minute reaction period will begin.

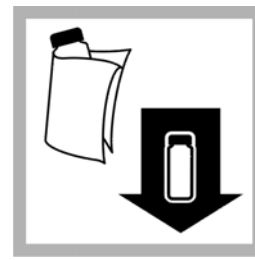
5. Invert the Ampul 48–52 times as the timer counts down.

6. When the timer expires, start the timer again.

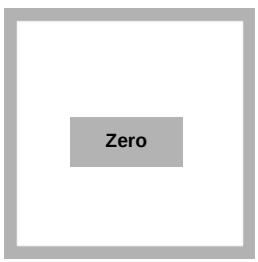
A five-minute reaction period will begin. An amber color will develop if nitrate is present.



7. Blank Preparation: When the timer expires, fill a round sample cell with 10 mL of sample.



8. Wipe the blank and insert it into the cell holder.

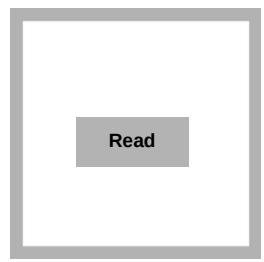


9. **ZERO** the instrument.

The display will show:

0.0 mg/L NO_3^-–N

10. Within two minutes after the timer expires, wipe the Ampul and insert it into the cell holder.



11. **READ** the results in mg/L NO_3^-–N.

Interferences

Table 225 Interfering substances

Interfering substance	Interference level
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration (see *Seawater calibration*).
Ferric iron	Interferes at all levels
Nitrite	Interferes at all levels Compensate for nitrite interference as follows: **1.** Add 30-g/L Bromine Water[1] drop-wise to the sample until a yellow color remains. **2.** Add one drop of 30-g/L Phenol Solution[1] to destroy the color. **3.** Proceed with Step 2 of the test. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

[1] See *Optional reagents and apparatus*.

Seawater calibration

Chloride concentrations above 100 mg/L will cause low results. To perform this test in water with high interference level, calibrate the water using standards spiked to the same chloride concentrations as the required samples. To prepare calibration standards containing 1.0, 3.0, 5.0 and 10.0 mg/L nitrate as NO_3–N:

1. Prepare a 1 L volume of chloride water that matches the concentration of the samples, using the following equation:

   c. Add necessary Chloride concentration (g/L) x (1.6485) = g of ACS grade NaCl to 1 L of deionized water.

   Note: *18.8 g/L is a typical seawater chloride concentration.*

   d. Mix this solution thoroughly to make sure that it is a homogeneous solution. Use this water as the dilution water instead of the deionized water when preparing the nitrate standards.

2. Use Class A glassware or a Tensette Pipet to pipet 1.0, 3.0, 5.0, and 10.0 mL of the 100 mg/L Nitrogen-Nitrate as NO_3^-–N (NIST) Standard Solution (Catalog Number 194749) into four different 100 mL Class A volumetric flasks.
3. Dilute to the mark with the prepared chloride water. Mix thoroughly.
4. Use the prepared chloride water for the 0-mg/L nitrate as NO_3–N standard.

Sample collection, preservation and storage

- Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (H_2SO_4)* per liter and store at 4 °C. The results are reported as total nitrate and nitrite.
- Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution*. Do not use mercury compounds as preservatives.

* See *Optional reagents and apparatus*.

- Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Nitrate Nitrogen Standard,100-mg/L NO_3^-–N
- TenSette Pipet and Pipet Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **Options>More>Standard Additions** from the instrument menu.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution bottle.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
6. Follow the *Cadmium reduction method for powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)
Required for accuracy check:

- 500 mg/L Nitrate Nitrogen Ampule Standard Solution
- Ampule breaker
- TenSette Pipet and Pipet Tips
- Mixing cylinder, 50-mL (3)

1. Fill three mixing cylinders each with 50-mL of sample and spike with 0.1 mL, 0.2 mL and 0.3 mL of 500 mg/L Nitrate Nitrogen Ampule Standard Solution.
2. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
3. Analyze each standard addition sample as described in the *Cadmium reduction method for AccuVac® Ampuls*.
4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery. Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Standard Solution Method
Required for accuracy check:

- 5.0-mg/L Nitrate Nitrogen Standard Solution (prepared)
- 100-mg/L Nitrate Nitrogen Standard
- Deionized water
- 100-mL volumetric flask

- 5-mL Volumetric pipet and pipet filler

  OR

- TenSette Pipet and Pipet Tips

1. Prepare a 5.0-mg/L nitrate nitrogen standard solution as follows:
   a. Pipet 5.0 mL of 100-mg/L Nitrate Nitrogen Standard, into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
2. Use this 5.0 mg/L nitrate nitrogen standard solution in place of the sample. Follow the *Cadmium reduction method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
353	5.0 mg/L NO_3^-–N	4.8–5.2 mg/L NO_3^-–N	0.04 mg/L NO_3^-–N
359	5.0 mg/L NO_3^-–N	4.6–5.4 mg/L NO_3^-–N	0.05 mg/L NO_3^-–N

Summary of method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. Test results are measured at 400 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitraVer® 5 Nitrate Reagent Powder Pillows (for 10 mL sample)	1	100/pkg	2106169
OR			
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1	25/pkg	2511025

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402
Stopper, Neoprene, solid, size #1	2	12/pkg	1480801

Required apparatus (AccuVac)

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606

Recommended standards

Description	Unit	Catalog number
Mixed Parameter Drinking Water Standard, for F, NO_3–N, PO_4, SO_4	500 mL	2833049
Nitrate Nitrogen Standard Solution, 100-mg/L NO_3^-–N	500 mL	194749
Nitrate Nitrogen Standard Solution, 500 mg/L NO_3–N, 10-mL ampules	16/pkg	1426010
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule breaker for 10 mL ampules	each	2196800
Bromine Water, 30-mg/L	29 mL	221120
Cylinder, mixing, 50 mL	each	2088641
Flask, volumetric, 100-mL	each	1457442
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, Class A, 5.0 mL	each	1451537
Pipet, volumetric, Class A, 1.0 mL	each	1451535
Pipet, volumetric, Class A, 3.0 mL	each	1451503
Pipet, volumetric, Class A, 10.0 mL	each	1451538
Pipet Filler, safety bulb	each	1465100
Phenol Solution, 30-g/L	29 mL	211220
5.0 N Sodium Hydroxide Standard Solution	1 L	245053
Sulfuric Acid, concentrated	500 mL	97949

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Edition 7

Nitrate

DOC316.53.01072

UV Screening Method[1]

Method 10049

0.1 to 10.0 mg/L NO_3^-–N

Scope and Application: For the screening of uncontaminated natural and potable water supplies containing low concentrations of organic matter

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*, part 4500-NO_3^-B.

Test preparation

How to use instrument-specific information

The instrument specific information table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 226 Instrument-specific information

Instrument	Sample cell	Adapter
DR 6000	4822800	A23618
DR 5000	4822800	A23618

Before starting the test:

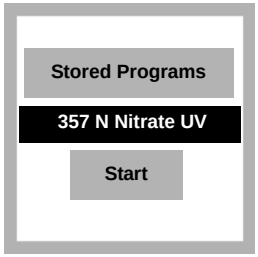
Turbid samples must be filtered prior to analysis.
This method is very sensitive to small differences in the measurement wavelength. For best results, use the Standard Adjust feature (as described in *Accuracy check*, *Standard solution method*) to optimize results on each instrument.

Collect the following items:

Description	Quantity
Hydrochloric Acid Standard Solution, 1.0 M	1 mL
Water, deionized	10 mL
Beaker, 100-mL	1
Sample Cells, 1-cm quartz	2

See *Consumables and replacement items* for reorder information.

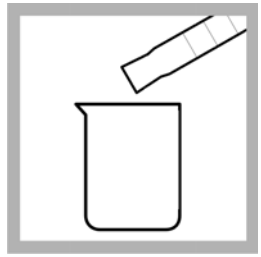
UV screening method



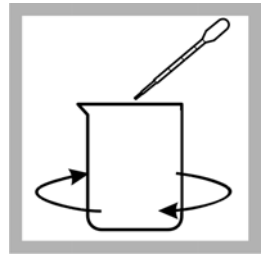
1. Select the test.

Insert an adapter if required.

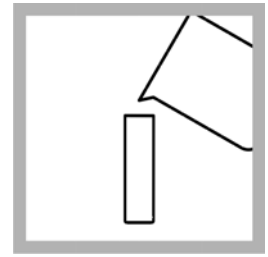
Refer to the user manual for orientation.



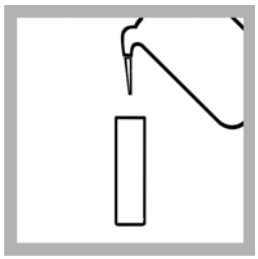
2. Prepared Sample: Collect 50 mL of clear sample in a 100-mL beaker.



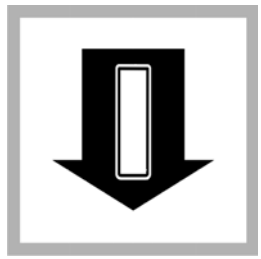
3. Add 1 mL of 1.0 N Hydrochloric Acid Standard Solution to the beaker and swirl to mix.



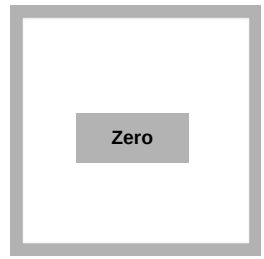
4. Rinse and fill a 1-cm quartz sample cell with sample. Discard the excess.



5. Blank Preparation: Fill another 1-cm quartz sample cell with deionized water.



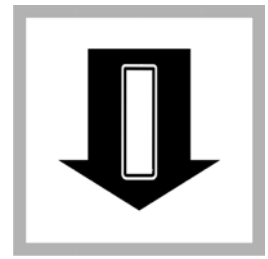
6. Insert the blank into the cell holder and close the lid. For the DR 5000, the transparent face of the cell should face the user. For the DR 6000, the transparent face of the cell should face right.



7. ZERO the instrument.

The display will show:

0.0 mg/L NO_3^-–N



8. Insert the prepared sample into the cell holder.

The instrument will read the sample at 220 and 275 nm. When finished, the display will show the sample nitrate nitrogen concentration.

Interferences

Table 227 Interfering substances

Interfering substance	Interference level
Chlorate	May interfere
Chromium	All levels
Dissolved organic matter	All levels
Nitrite	All levels
Surfactants	All levels
Suspended particulate matter	Remove using filtration.

Sample collection, preservation and storage

- More reliable results are obtained when samples are analyzed as soon as possible after collection.
- If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C.
- To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (H_2SO_4)* per liter and store at 4 °C.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Deionized water
- 100 mL volumetric flask
- 5-mL Volumetric pipet and pipet filler
- 100 mg/L Nitrate Nitrogen Standard

  OR

- 5 mg/L Nitrate Nitrogen Voluette Ampul Standard

1. Prepare a 5.0 mg/L Nitrate Nitrogen standard solution as follows:
   a. Pipet 5.0 mL of 100 mg/L Nitrate Nitrogen Standard, into a 100-mL volumetric flask.
   b. Dilute to volume with deionized water. Insert a stopper and invert to mix. Alternatively, open a 5 mg/L Nitrate Nitrogen Voluette Ampul Standard.
2. Use the 5.0 mg/L nitrate nitrogen standard solution in place of the sample. Follow the *UV screening method* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
357	5.0 mg/L NO_3^-–N	4.9–5.1 mg/L NO_3^-–N	0.1 mg/L NO_3^-–N

Summary of method

The UV nitrate direct screening method offers rapid determination of nitrate. Because both nitrate and organic constituents absorb at 220 nm and nitrate does not absorb at 275 nm, the second reading at 275 nm is used to correct for the absorbance attributed to organic matter. Although this

* See *Optional reagents and apparatus*.

method is useful for monitoring nitrate, it is not recommended for samples containing high concentrations of organics. Adding hydrochloric acid prevents interference from hydroxide or carbonate concentrations up to 1000 mg/L $CaCO_3$.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hydrochloric Acid Standard Solution, 1.0 M	1 mL	1 L	2321353
Water, deionized	10 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 100-mL	1	each	50042H
Sample cell with cap, 1-cm quartz, matched pair	2	2/pkg	4822800

Recommended standards

Description	Unit	Catalog number
Nitrate Nitrogen Standard Solution, 100-mg/L NO_3^-N	500 mL	194749
Nitrate Nitrogen Voluette Standard, 5 mg/L NO3-N, 10 mL ampuls	16/pkg	2557810
Ampule Breaker - Voluette	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Aspirator, Nalgene vacuum pump	each	213100
Dropper, 0.5 and 1.0 mL	20/pkg	2124720
FIlter holder, 47-mm	each	1352900
Filter membrane, 47-mm, 0.45 micron	100/pkg	1353000
Flask, filtering, 500-mL	each	54649
Flask, volumetric, 100-mL	each	1457442
Pipet Filler, safety bulb	each	1465100
Pipet, serological, 2-mL	each	53236
Pipet, volumetric, Class A, 5.00-mL	each	1451537
Stopper, No. 7, one hole	6/pkg	211907
Sulfuric Acid, concentrated, ACS	500 mL	97949
Tubing, rubber latex	12 ft	56019

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Nitrite

DOC316.53.01075

Ferrous Sulfate Method[1] **Method 8153**

HR (2 to 250 mg/L NO_2^-) **Powder Pillows**

Scope and Application: For cooling systems

[1] Adapted from McAlpine, R. and Soule, B., *Qualitative Chemical Analysis*, New York, 476, 575 (1933)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 228 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

After adding the reagent, a greenish-brown color will develop if nitrite is present.

Collect the following items:

Description	Quantity
NitriVer® 2 Nitrite Reagent Powder Pillows	1
Deionized water	varies
Stopper, Neoprene, solid # 1	2
Sample Cells (see *Instrument-specific information*)	2

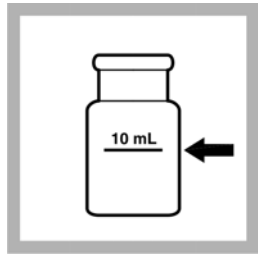
See *Consumables and replacement items* for reorder information.

Ferrous sulfate method for powder pillows

1. Select the test.

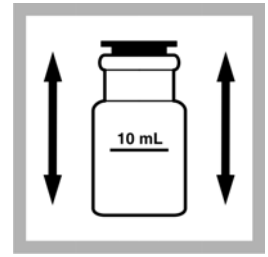
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a sample cell with 10 mL of sample.

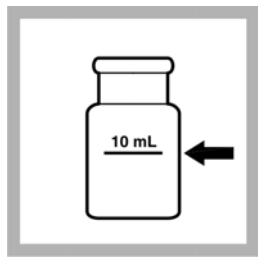
3. Prepared Sample: Add the contents of one NitriVer® 2 Nitrite Reagent Powder Pillow.



4. Insert the stopper and shake to dissolve.

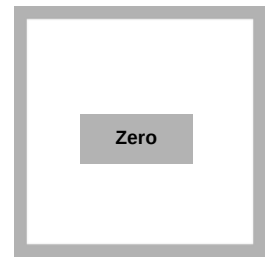
5. Start the instrument timer.

A ten-minute reaction period will begin. To prevent low results, leave the sample on a flat surface and **do not disturb it during the reaction period.**



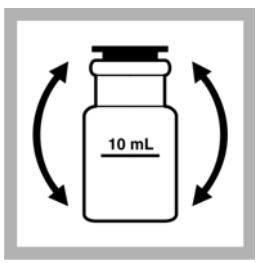
6. Blank Preparation: Fill a second sample cell with 10 mL of sample.

7. Wipe the blank and insert it into the cell holder.



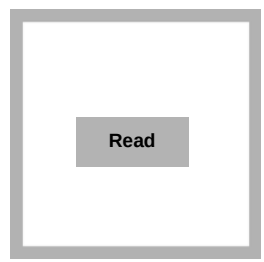
8. ZERO the instrument.

The display will show: 0 mg/L NO_2^-



9. After the timer expires, cap and **gently** invert the prepared sample twice. Avoid excessive mixing or low results may occur.

10. Wipe the prepared sample and insert it into the cell holder.



11. READ the results in mg/L NO_2^-.

Interferences

This test does not measure nitrates nor is it applicable to glycol-based samples. Dilute glycol-based samples and follow the Low Range Nitrite procedure, Method 8507.

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles.

The following storage instructions are necessary when prompt analysis is impossible. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. Do not use acid preservatives.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Preparing nitrite standards is difficult. Use the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*. Prepare a 200-mg/L standard using Sodium Nitrite, ACS*, reagent grade.
2. Use the 200-mg/L solution in place of the sample. Follow the *Ferrous sulfate method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
373	Standard: 200 mg/L NO_2^-	191–209 mg/L NO_2^-	1.4 mg/L NO_2^-

Summary of method

The method uses ferrous sulfate in an acidic medium to reduce nitrite to nitrous oxide. Ferrous ions combine with the nitrous oxide to form a greenish-brown complex in direct proportion to the nitrite present. Test results are measured at 585 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitriVer® 2 Nitrite Reagent Powder Pillows	1	100/ pkg	2107569

* See *Optional reagents and apparatus*.

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
Stopper, Neoprene, solid #1	2	12/pkg	1480801
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, Analytical, 80 g capacity	each	2936701
Handbook, Standard Methods for the Examination of Water and Wastewater	each	2270800
Water, deionized	4 L	27256
Sodium Nitrite, ACS	454 g	245201

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Edition 7

Nitrite

DOC316.53.01073

Diazotization Method **Method 10019**

LR (0.003 to 0.500 mg/L NO_2^-–N) **Test ‘N Tube™ Vials**

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 229 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in cell compartment #2 before performing this test.

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

Description	Quantity
Light Shield (see *Instrument-specific information*)	1
Test ‘N Tube™ NitriVer® 3 Nitrite Reagent Set	1
Pipet, TenSette®, 1.0 to 10.0 mL, plus tips	1

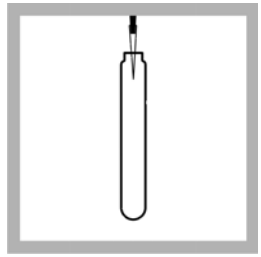
See *Consumables and replacement items* for reorder information.

Diazotization method, TNT

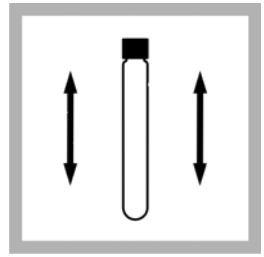
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a Test 'N Tube NitriVer® 3 Nitrite vial with 5 mL of sample.



3. Prepared Sample: Cap and shake to dissolve the powder.

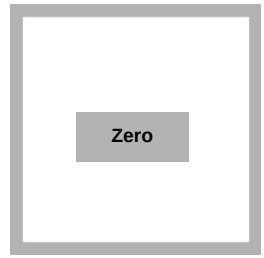
A pink color will develop if nitrite-nitrogen is present.

4. Start the instrument timer.

A 20-minute reaction period will begin.

5. Blank Preparation: When the timer expires, fill an empty Test 'N Tube™ vial with 5 mL of sample.

6. Wipe the blank and insert it into the 16-mm round cell holder.



7. **ZERO** the instrument.

The display will show: 0.000 mg/L NO_2^-–N

8. Wipe and insert the prepared sample cell into the 16-mm round cell holder.

READ the results in mg/L NO_2^-–N.

Interferences

Table 230 Interfering substances

Interfering substance	Interference level
Antimonous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation

Table 230 Interfering substances (continued)

Interfering substance	Interference level
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interfere at all levels

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Store the samples at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours.
- Warm the sample to room temperature before running the test.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Preparing nitrite standards is difficult. Use the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500-NO_2^- B. Prepare a 0.300-mg/L standard. Use this solution in place of the sample. Follow the *Diazotization method, TNT* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
345	0.300 mg/L NO_2^-–N	0.294–0.306 mg/L NO_2^-–N	0.003 mg/L NO_2^-–N

Summary of method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Test 'N Tube™ NitriVer® 3 Nitrite Reagent Set	1	50/pkg	2608345

Required apparatus

Description	Quantity	Unit	Catalog number
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 1970010	varies	50/pkg	2199796

Recommended standards, reagents and apparatus

Description	Unit	Catalog number
Handbook, Standard Methods for the Examination of Water and Wastewater	each	2270800
Balance, Analytical, 80 g capacity	each	2936701
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
Sodium Nitrite, ACS	454 g	245201
Water, deionized	4 L	27256

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Edition 7

Nitrite

DOC316.53.01074

USEPA[1] Diazotization **Method 8507**

LR (0.002 to 0.300 mg/L NO_2^-–N) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater

[1] USEPA approved for wastewater analysis, Federal Register, 44(85), 25505 (May 1, 1979)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 231 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
NitriVer® 3 Nitrite Reagent Powder Pillows	1
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
NitriVer® 3 Nitrite Reagent AccuVac® Ampul.	1
Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1

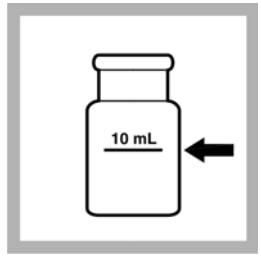
See *Consumables and replacement items* for reorder information.

Diazotization method for powder pillows

1. Select the test.

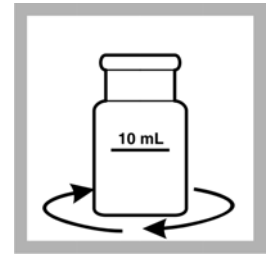
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill a sample cell with 10 mL of sample.

3. Prepared Sample: Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow.

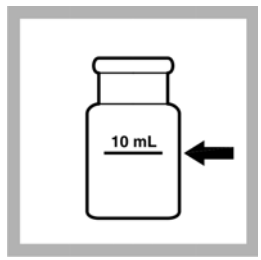


4. Swirl to dissolve.

A pink color will develop if nitrite is present.

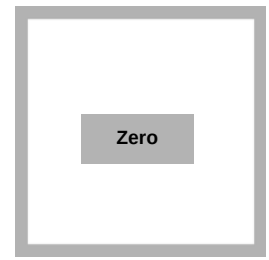
5. Start the instrument timer.

A 20-minute reaction period will begin.



6. Blank Preparation: When the timer expires, fill a second sample cell with 10 mL of sample.

7. Wipe the blank and insert it into the cell holder.

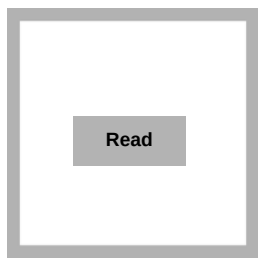


8. **ZERO** the instrument.

The display will show:

0.000 mg/L NO_2^-–N

9. Wipe the prepared sample and insert it into the cell holder.



10. **READ** the results in mg/L NO_2^-–N.

Diazotization method for AccuVac® Ampuls

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

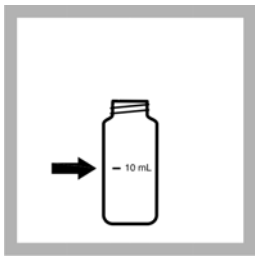
2. Prepared Sample: Collect at least 40 mL of sample into a 50-mL beaker.

Fill a NitriVer 3 Nitrite AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills.

3. Invert the Ampul several times to mix. A pink color will develop if nitrite is present.

4. Start the instrument timer.

A 20-minute reaction period will begin.



5. Blank Preparation: When the timer expires, fill a sample cell with 10 mL of sample.

6. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show: 0.000 mg/L NO_2^-–N.

7. Wipe the Ampul and insert it into the cell holder.

READ the results in mg/L NO_2^-–N.

Interferences

Table 232 Interfering substances

Interfering substance	Interference level
Antimonous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interferes at all levels

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours.
- Warm to room temperature before running the test.
- Do not use acid preservatives.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Preparing nitrite standards is difficult. Use the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500—NO_2-B. Prepare a 0.150-mg/L standard.
2. Use the 0.150 mg/L solution in place of the sample. Follow the *Diazotization method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, select **Options>More>Standard Adjust** from the instrument menu.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
371	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
375	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N

Summary of method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitriVer® 3 Nitrite Reagent Powder Pillows	1	100/pkg	2107169
OR			
NitriVer® 3 Nitrite Reagent AccuVac® Ampul	1	25/pkg	2512025

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards, reagents and apparatus

Description	Unit	Catalog number
Balance, Analytical, 80 g capacity	each	2936701
AccuVac ampules, for blanks	25/pkg	2677925
AccuVac Snapper	each	2405200
AccuVac Drainer	each	4103600
Handbook, Standard Methods for the Examination of Water and Wastewater	each	2270800
Sodium Nitrite, ACS	454 g	245201
Water, deionized	4 L	27256

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Edition 7

Nitrite

DOC316.53.01178

Ceric Acid Titration Method

Method 8351

100 to 2500 mg/L as $NaNO_2$

Digital Titrator

Scope and Application: For cooling tower waters.

Test preparation

Before starting the test:

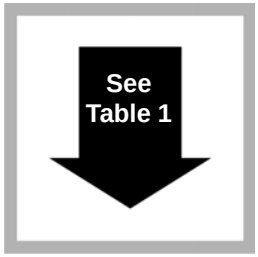
For added convenience when stirring, use the TitraStir® stirring apparatus.
A pipet is recommended for measuring the sample volume when the volume is less than 10 mL.

Collect the following items:

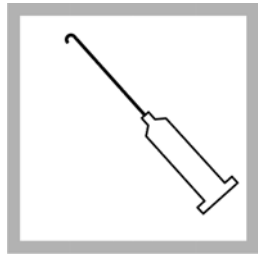
Description	Quantity
Ferroin Indicator Solution	1 bottle
Sulfuric Acid Standard Solution, 5.25 N	1 bottle
Ceric Standard Solution Titration Cartridge, 0.5 N	1 cartridge
Digital titrator	1
Delivery tube for digital titrator	1
Graduated cylinder	1
Erlenmeyer flask, 125-mL	1

See *Consumables and replacement items* for reorder information.

Nitrite



1. Select a sample volume from the *Range-specific information* table.



2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator.

3. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

4. Use a graduated cylinder or pipet to measure the sample volume from the *Range-specific information* table in a 125 mL Erlenmeyer flask.

5. Dilute to approximately 75 mL with deionized water.

6. Add five drops of 5.25 N Sulfuric Acid Standard Solution to the flask. Swirl to mix.

7. Add one drop of Ferroin Indicator Solution to the flask. Swirl to mix.

8. Place the delivery tube into the solution and swirl the flask. Turn the knob on the titrator to add titrant to the solution. Continue to swirl the flask and add titrant until the color changes from orange to pale blue.

Write down the number of digits displayed on the counter.

Nitrite (continued)

9. Use the multiplier in the *Range-specific information* table to calculate the concentration:

digits x multiplier = mg/L sodium nitrite ($NaNO_2$)

Example: 25 mL of sample was titrated and 250 digits were used to reach the endpoint. The concentration is 250 x 0.86 = 215 mg/L as $NaNO_2$

Table 233 Range-specific information

Range (mg/L as $NaNO_2$)	Sample volume (mL)	Multiplier
100–400	25	0.86
400–800	10	2.15
800–1500	5	4.31
1500–2500	2	10.78

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Prompt analysis is recommended.

If prompt analysis is not possible, store samples for 24 to 48 hours at 4 °C (39 °F) or lower. Warm to room temperature before analysis. Do not use acid preservatives.

Accuracy check

Standard solution method
Required for accuracy check:

- Sodium Nitrite, ACS
- 1000-mL volumetric flask, Class A

1. Prepare a 1000-mg/L sodium nitrite standard solution as follows. Add 1.000 gram of sodium nitrite to the volumetric flask. Add deionized water to the mark and mix fully.
2. Add 5.0 mL of the solution to an Erlenmeyer flask. Dilute to approximately 75 mL with deionized water and mix fully.
3. Add the sulfuric acid and Ferroin indicator. Swirl to mix.
4. Titrate the standard to the end point with the titration cartridge and calculate the result. The result should be close to 1000 mg/L as $NaNO_2$.

Standardization of ceric standard solution
The normality of the ceric standard solution will sometimes decrease over time. Before use, verify the normality with the following procedure. This standardization should be done monthly.

1. Use a graduated cylinder or pipet to measure 50 mL of deionized water into a 125-mL Erlenmeyer flask.
2. Add 5 mL of 19.2 N Sulfuric Acid Standard Solution. Swirl to mix.
3. Insert a clean delivery tube into a Ceric Standard Titration Cartridge.
4. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob until a few drops of titrant are expelled. Reset the counter to zero and wipe the tip.
5. Place the delivery tube tip into the solution. While swirling the flask, add 200 digits of Ceric Standard.
6. Insert a clean delivery tube into a 0.200 N Sodium Thiosulfate Titration Cartridge.
7. Hold the Digital Titrator with the cartridge tip pointing up. Turn the delivery knob until a few drops of titrant are expelled. Reset the counter to zero and wipe the tip.
8. Place the delivery tube tip into the solution. While swirling the flask, titrate with the sodium thiosulfate from an intense yellow color to a faint yellow color. Record the number of digits required. This step should require about 400–450 digits of titrant.

9. Add one drop of Ferroin Indicator Solution. Swirl to mix. The solution will turn a faint blue.
10. Continue titrating with the Sodium Thiosulfate Standard Solution from a faint blue to orange color. Record the number of digits required.
11. Divide the number of digits by 500 to calculate the correction factor (number of digits , 500 = correction factor).
12. Multiply the mg/L sodium nitrite from the titration procedure by the correction factor to obtain the correct sodium nitrite concentration.

Summary of method

Sodium nitrite is titrated with tetravalent cerium ion, a strong oxidant, in the presence of ferroin indicator. After the cerium oxidizes the nitrite, the indicator is oxidized and causes a color change from orange to pale blue. The concentration of sodium nitrite is proportional to the amount of titrant.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ceric Standard Solution Titration Cartridge, 0.5 N	1 pillow	each	2270701
Ferroin Indicator Solution	1 pillow	29 mL DB	181233
Sulfuric Acid Standard Solution, 5.25 N	1 pillow	100 mL MDB	244932

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Digital Titrator	1	each	1690001
Flask, Erlenmeyer, graduated, 125-mL	1	each	50543
Cylinder, graduated, 25-mL	1	each	50840
Delivery tubes w/ 180° hook	1	each	1720500
Delivery tubes w/ 90° hook	1	each	4157800

Recommended standards

Description	Unit	Catalog number
Sodium Nitrite, ACS	454 g	245201
Sodium Thiosulfate Titration Cartridge, 0.200 N	each	2267501
Sulfuric Acid Standard Solution, 19.2 N	100 mL	203832

Optional reagents and apparatus

Description	Unit	Catalog number
Stir bar, octagonal 28.6 mm x 7.9 mm	each	2095352
TenSette Pipet, 1.0 to 10.0 mL	each	1970010
TitraStir Stir Plate, 115 VAC	each	1940000
TitraStir Stir Plate, 230 VAC	each	1940010
Water, deionized	500 mL	27249
Thermometer -10 to 225 °C	405 mm	2635700
Volumetric flask, 100 mL	each	1457453
Volumetric pipet, 5 mL	each	1451537
Sampling bottle, 250 mL	each	2087076
Analytical balance	each	2936701
Weighing papers	500/pkg	1473800

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Edition 7

Nitrogen, Ammonia

DOC316.53.01078

USEPA[1] Nessler Method[2]

Method 8038

0.02 to 2.50 mg/L NH_3–N

Scope and Application: For water, wastewater and seawater; distillation is required for wastewater and seawater; USEPA accepted for wastewater analysis (distillation required); see *Distillation* in this procedure.

[1] USEPA accepted for wastewater analysis (distillation required)

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater 4500-NH_3 B & C*.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 234 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Nessler Reagent contains mercuric iodide. Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste. Do not pour these solutions down the drain. Refer to a current MSDS for safe disposal and handling instructions.

When dispensing reagent from a dropper bottle, hold the bottle vertically. Do not hold the bottle at an angle.

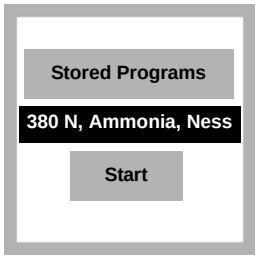
If the Flow Cell is used, periodically clean the cells by pouring a few sodium thiosulfate pentahydrate crystals in to the cell funnel. Flush with enough deionized water to dissolve. Rinse the cell thoroughly.

Collect the following items:

Description	Quantity
Ammonia Nitrogen Reagent set	1
Deionized Water	25 mL
Graduated Mixing Cylinders	2
Sample Cells, 1-inch, 10-mL	2
Serological Pipet, 1-mL	1

See *Consumables and replacement items* for reorder information.

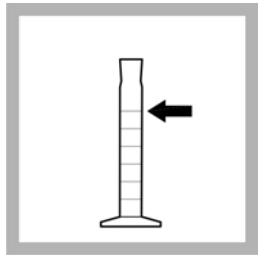
Nessler method



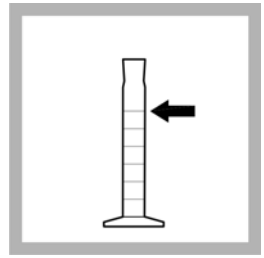
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

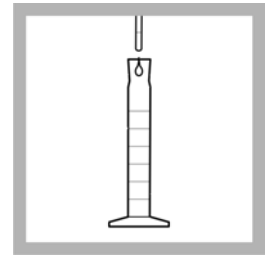
Refer to the user manual for orientation.



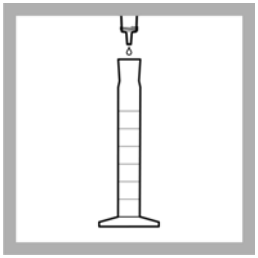
2. Prepared Sample: Fill a 25-mL mixing graduated cylinder to the 25-mL mark with sample.



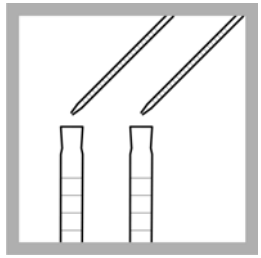
3. Blank Preparation: Fill a 25-mL mixing graduated cylinder to the 25-mL mark with deionized water.



4. Add three drops of Mineral Stabilizer to each cylinder. Stopper and invert several times to mix.



5. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Stopper and invert several times to mix.



6. Pipet 1.0 mL of Nessler Reagent into each cylinder. Stopper and invert several times to mix.

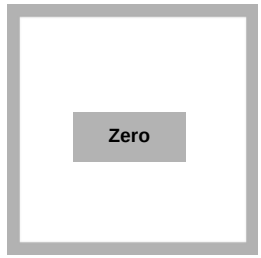
7. Start the instrument timer.

A one-minute reaction period will begin.

8. Pour 10 mL of each solution into a sample cell.

Nessler method (continued)

9. When the timer expires, insert the blank into the cell holder.

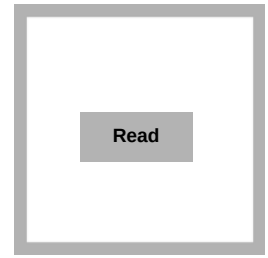


10. **ZERO** the instrument.

The display will show:

0.00 mg/L NH_3 –N

11. Wipe the prepared sample and insert it into the cell holder.



12. **READ** the results in mg/L NH_3 –N.

Interferences

Table 235 Interfering substances

Interfering substance	Interference level
Chlorine	Remove residual chlorine from a 250 mL sample by adding 1 drop of sodium thiosulfate for each mg/L chlorine (Cl_2). Sodium arsenite can be used instead of sodium thiosulfate. See *Sample collection, preservation and storage*.
Hardness	A solution containing a mixture of 500 mg/L $CaCO_3$ and 500 mg/L Mg as $CaCO_3$ does not interfere. If the hardness concentration exceeds these concentrations, add extra Mineral Stabilizer.
Iron	Interferes at all levels by causing turbidity with Nessler Reagent.
Seawater	May be analyzed by adding of 1.0 mL (27 drops) of Mineral Stabilizer to the sample before analysis. This complexes the high magnesium concentrations found in sea water, but the sensitivity of the test is reduced by 30 percent due to the high chloride concentration. For best results, perform a calibration, using standards spiked to the equivalent chloride concentration or distill the sample as described below.
Sulfide	Interferes at all levels by causing turbidity with Nessler Reagent.
Glycine, various aliphatic and aromatic amines, organic chloramines, acetone, aldehydes and alcohols	May cause greenish or other off colors or turbidity. Distill the sample if these compounds are present.

Sample collection, preservation and storage

- Collect samples in clean glass or plastic bottles.
- If chlorine is present, add one drop of 0.1 N Sodium Thiosulfate* for each 0.3 mg/L Cl_2 in a 1-liter sample.
- Preserve the sample by reducing the pH to 2 or less with sulfuric acid (at least 2 mL/L). Store at 4 °C (39 °F) or less.
- Preserved samples may be stored up to 28 days.
- Warm samples to room temperature and neutralize with 5 N Sodium Hydroxide* before analysis.

* See *Optional reagents and apparatus*.

- Correct the test result for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Nitrogen Ammonia Voluette® Ampule Standard, 50-mg/L NH_3–N
- Ampule breaker
- TenSette Pipet and Pipet Tips
- 25-mL Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample in three mixing cylinders.
6. Follow the *Nessler method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Nitrogen Ammonia Standard solution 1-mg/L NH_3–N

1. Use a 1-mg/L Nitrogen Ammonia Standard solution in place of the sample. Follow the *Nessler method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Distillation

1. Measure 250 mL of sample into a 250-mL graduated cylinder and pour into a 400-mL beaker. Destroy chlorine, if necessary, by adding 1 drop of Sodium thiosulfate Solution 0.1 N per mg/L Cl_2.
2. Add 25 mL of Borate Buffer Solution and mix. Adjust the pH to about 9.5 with 1 N sodium hydroxide solution. Use a pH meter.
3. Set up the General Purpose Distillation Apparatus as shown in the *Distillation Apparatus Manual*. Pour the solution into the distillation flask. Add a stir bar.

4. Use a graduated cylinder to measure 25 mL of deionized water into a 250-mL Erlenmeyer flask. Add the contents of one Boric Acid Powder Pillow. Mix thoroughly. Set the flask under the distillation apparatus drip tube. Elevate the flask so the end of the tube is immersed in the solution.
5. Turn on the heater power switch. Set the stir control to 5 and the heat control to 10. Turn on the water and adjust to maintain a constant flow through the condenser.
6. Turn off the heater after collecting 150 mL of distillate. Immediately remove the collection flask to avoid sucking solution into the still. Measure the distillate to ensure 150 mL was collected (total volume = 175 mL).
7. Adjust the pH of the distillate to about 7 with 1 N sodium hydroxide. Use a pH meter.
8. Pour the distillate into a 250-mL volumetric flask; rinse the Erlenmeyer with deionized water. Add the rinsings to the volumetric flask. Dilute to the mark. Stopper. Mix thoroughly. Analyze as described above.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
380	DR 5000	1.00 mg/L NH_3–N	0.99–1.01 mg/L NH_3–N	0.02 mg/L NH_3–N2

Summary of method

The Mineral Stabilizer complexes hardness in the sample. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonia and certain other amines. A yellow color is formed proportional to the ammonia concentration. Test results are measured at 425 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ammonia Nitrogen Reagent Set, includes:	—	—	2458200
Nessler Reagent	2 mL	500 mL	2119449
Mineral Stabilizer	6 drops	50 mL SCDB	2376626
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	2376526
Water, deionized	25 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, mixing, 25-mL	2	each	2088640
Pipet, serological, 1-mL	1	each	919002
Pipet Filler, safety bulb	1	each	1465100

Recommended standards and apparatus

Description	Unit	Catalog number
Nitrogen, Ammonia Standard Solution, 1-mg/L NH_3–N	500 mL	189149
Nitrogen, Ammonia Standard Solution, 10-mL Voluette® Ampule, 50-mg/L NH_3–N	16/pkg	1479110
Pipet, TenSette® 0.1 - 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Wastewater, Effluent Inorganics, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249

Optional reagents and apparatus

Description	Unit	Catalog number
Distillation Apparatus, General	each	2265300
Heater and Support Apparatus, 115 VAC, 60 Hz	each	2274400
Heater and Support Apparatus, 230 VAC, 50 Hz	each	2274402
Pour-Thru Cell Kit for DR 2800	each	5940400
Pour-Thru Cell Module for DR 5000	each	LZV479
Ampule breaker, Voluette	each	2196800
Sodium Thiosulfate, 0.1 N	100 mL	32332
Sodium Hydroxide, 5 N	100 mL	245032

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Edition 7

Nitrogen, Ammonia

DOC316.53.01077

Salicylate Method[1] **Method 8155**

0.01 to 0.50 mg/L NH_3–N **Powder Pillows**

Scope and Application: For water, wastewater and seawater

[1] Adapted from *Clin. Chim. Acta., 14*, 403 (1966)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 236 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

A green color will develop if ammonia nitrogen is present.

Dispose of reacted solutions according to local, state and federal regulations. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.

Collect the following items:

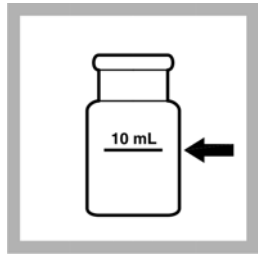
Description	Quantity
Ammonia Cyanurate Reagent pillows	2
Ammonia Salicylate Reagent pillows	2
Sample Cells (see *Instrument-specific information*)	2
Stopper for sample cells	2
See *Consumables and replacement items* for reorder information.	

Salicylate method for powder pillows

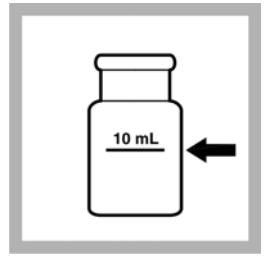
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Prepared Sample: Fill a sample cell to the 10-mL mark with sample.



3. Blank Preparation: Fill a second sample cell to the 10-mL mark with deionized water.

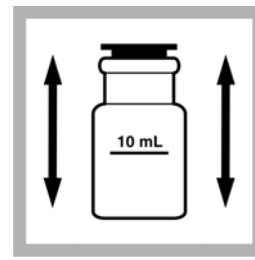
4. Add the contents of one Ammonia Salicylate Powder Pillow to each cell.

5. Insert the stopper or cap the cell and shake to dissolve.

6. Start the instrument timer.

A three-minute reaction period will begin.

7. When the timer expires, add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each cell.



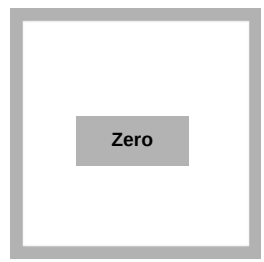
8. Stopper or cap and shake to dissolve.

9. Start the instrument timer.

A 15-minute reaction period will begin.

A green color will develop if ammonia-nitrogen is present.

10. When the timer expires, wipe the blank and insert it into the cell holder



11. ZERO the instrument.

The display will show: 0.00 mg/L NH_3–N.

12. Wipe the sample and insert it into the cell holder.

READ the results in mg/L NH3–N.

Interferences

Table 237 Interfering substances

Interfering substance	Interference level
Calcium	Greater than 1000 mg/L as $CaCO_3$
Iron	All levels. Correct for iron interference as follows: **1.** Determine the amount of iron present in the sample by following one of the Iron, Total, procedures. **2.** Add the same iron concentration to the ammonia-free water in step 2. The interference will be successfully blanked out.
Magnesium	Greater than 6000 mg/L as $CaCO_3$
Monochloramine	Monochloramine present in chloraminated drinking water interferes directly at all levels, giving high results. Use Method 10200, Free Ammonia and Monochloramine, to determine free ammonia in these sample matrices.
Nitrate	Greater than 100 mg/L as NO_3^-–N
Nitrite	Greater than 12 mg/L as NO_2^-–N
Phosphate	Greater than 100 mg/L as PO_4^{3-}–P
Sulfate	Greater than 300 mg/L as SO_4^{2-}
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: **1.** Measure about 350 mL of sample in a 500-mL Erlenmeyer flask[1]. **2.** Add the contents of one Sulfide Inhibitor Reagent[1] Powder Pillow. Swirl to mix. **3.** Filter the sample through a Folded Filter Paper[1] and Filter Funnel[1]. **4.** Use the filtered solution in step 3.
Other Substances	Less common interferences such as **hydrazine** and **glycine** will cause intensified colors in the prepared sample. **Turbidity** and **color** will give erroneous high values. Samples with severe interferences require distillation. Use the distillation procedure with the General Purpose Distillation Set.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.
- If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate* for each 0.3 mg/L Cl_2 in a one-liter sample.
- Adjust the pH to 2 or less with concentrated (about 2 mL per liter) Sulfuric Acid.
- Store samples at 4 °C or less. Samples preserved in this manner can be stored up to 28 days.
- Just before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution.
- Correct the test result for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Ammonia Nitrogen Standard Solution, 10-mg/L as NH_3–N
- TenSette Pipet and Pipet Tips
- 25-mL Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution.
5. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL and 0.6 mL of standard to three 25-mL portions of fresh sample in three mixing cylinders.
6. Follow the *Salicylate method for powder pillows* test procedure for each of the spiked samples, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Ammonia Nitrogen Standard Solution, 10-mg/L as NH_3–N
- TenSette Pipet, 0.1–1.0 mL and Pipet Tips

OR

- Ammonia Nitrogen Voluette® Standard Solution, 50-mg/L as NH_3–N
- Deionized water
- 100 mL Class A volumetric flask
- 4-mL Class A volumetric pipet

1. Prepare a 0.40 mg/L ammonia nitrogen standard solution as follows:
   - Dilute 4.00 mL of Ammonia Nitrogen Standard Solution, 10-mg/L to 100 mL with deionized water.

   OR

   - Use the TenSette® Pipet to dilute 0.8 mL of an Ammonia Nitrogen Voluette Standard Solution, 50-mg/L as NH_3–N, to 100 mL with deionized water
2. Use the 0.40-mg/L solution in place of the sample. Follow the *Salicylate method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
385	0.40 mg/L NH_3–N	0.38–0.42 mg/L NH_3–N	0.004 mg/L NH_3–N

Summary of method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ammonia Nitrogen Reagent Set for 10-mL samples (100 tests), includes:	—	—	2668000
Includes:			
(2) Ammonia Cyanurate Reagent Powder Pillows	2	100/pkg	2653199
(2) Ammonia Salicylate Reagent Powder Pillows	2	100/pkg	2653299

Recommended standards and apparatus

Description	Unit	Catalog number
Nitrogen, Ammonia Standard Solution, 10-mg/L NH_3–N	500 mL	15349
Nitrogen, Ammonia Standard Solution, 10-mL Voluette® Ampule, 50-mg/L NH_3–N	16/pkg	147910
Wastewater, Effluent Inorganics, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Pipet, TenSette® 0.1 - 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Flask, volumetric, Class A, 100 mL	each	1457442
Pipet, volumetric, Class A, 4.00 mL	each	1451504
Stopper, 18 mm	each	6/pkg
Water, deionized	4 L	27256
Sample cell, 10 mL square, matched pair	2/pkg	2495402

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25-mL	each	2088640

Optional reagents and apparatus

Description	Unit	Catalog number
Distillation Set, general purpose	each	2265300
Erlenmeyer Flask, 500 mL	each	50549
Filter Funnel, Analytical PP, 65 mm	each	108367
Filter Paper, folded, 12.5 cm	100/pkg	189457
Heater and support apparatus; 115 Vac, 60 Hz	each	2274400
Heater and support apparatus, 230 Vac, 50 Hz	each	2274402
Pipet, 2 mL Serological	each	53236
Ampule breaker, Voluette	each	2196800
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	245026
Sulfide Inhibitor Reagent Powder Pillow	100/pkg	241899
Sulfuric Acid, Conc	500 mL	97949

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Edition 7

Nitrogen, Ammonia

DOC316.53.01079

Salicylate Method **Method 10031**

HR (0.4 to 50.0 mg/L NH_3–N) **Test 'N Tube™ Vials**

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 238 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in cell compartment #2 before performing this test.
Small sample sizes (such as 0.1 mL) may not be representative of the entire sample. Mix the sample well before testing or repeat the test, sampling from different portions of the sample.
Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.
Dispose of reacted solutions according to local, state and federal regulations. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.
In some lab environments, airborne cross-contamination of the blank is possible. To avoid the transfer of ammonia, complete the preparation of the blank before opening or handling samples and standards. If the sample or standard containers have already been opened, move to a separate area of the lab to prepare the blank.

Collect the following items:

Description	Quantity
High Range Test 'N Tube AmVer™ Nitrogen Ammonia Reagent	2
Light Shield (see *Instrument-specific information*)	1
Funnel, micro (for adding reagent)	1
Pipet, TenSette®, 0.1–1.0 mL	1
Pipet Tips, for TenSette Pipet	varies

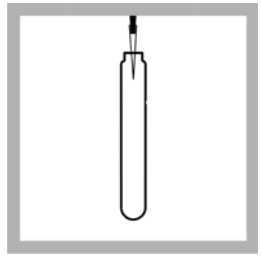
See *Consumables and replacement items* for reorder information.

Salicylate method, TNT

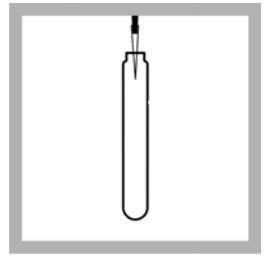
1. Select the test.

Insert an adapter or light shield if required (see *Instrument-specific information*).

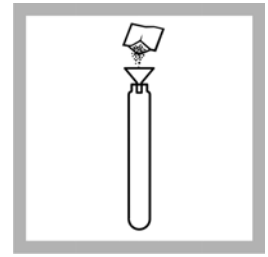
Refer to the user manual for orientation.



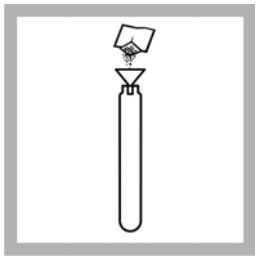
2. Prepared Sample: Add 0.1 mL of sample to one AmVer™ Diluent Reagent Test 'N Tube for High Range Ammonia Nitrogen.



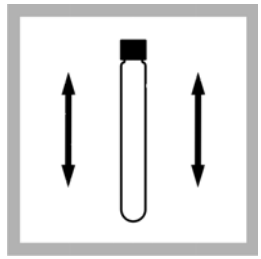
3. Blank Preparation: Add 0.1 mL of ammonia-free water to one AmVer™ Diluent Reagent Test 'N Tube for High Range Ammonia Nitrogen.



4. Add the contents of one Ammonia Salicylate Reagent Powder Pillow for 5 mL sample to each vial.



5. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each vial.

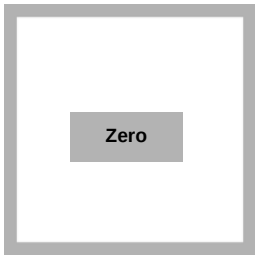


6. Cap the vials tightly and shake thoroughly to dissolve the powder.

7. Start the instrument timer.

A 20-minute reaction period will begin.

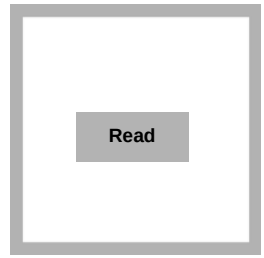
8.



9. **ZERO** the instrument.

The display will show: 0.0 mg/L NH_3–N

10.



11. **READ** the results in mg/L NH_3–N.

Interferences

Table 239 Interfering substances

Interfering substance	Interference level
Calcium	50,000 mg/L as $CaCO_3$
Glycine, hydrazine	Will cause intensified colors in the prepared sample.
Magnesium	300,000 mg/L as $CaCO_3$
Monochloramine	Monochloramine present in chloraminated drinking water interferes directly at all levels giving high results. Use Method 10200, Free Ammonia and Monochloramine, to determine free ammonia in these sample matrices.
Iron	Eliminate iron interference as follows: **1.** Determine the amount of iron present in the sample using one of the total iron procedures. **2.** Add the same iron concentration to the deionized water in step 3. The interference will then be successfully blanked out.
Nitrite	600 mg/L as NO_2^-–N
Nitrate	5000 mg/L as NO_3^-–N
Orthophosphate	5000 mg/L as PO_4^{3-}–P
pH	Acidic or basic samples should be adjusted to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution[1] for acidic samples and 1 N Hydrochloric Acid Standard Solution[1] for basic samples.
Sulfate	6000 mg/L as SO_4^{2-}
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: **1.** Measure about 350 mL of sample in a 500-mL Erlenmeyer flask. **2.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow[1]. Swirl to mix. **3.** Filter the sample through folded filter paper[1]. Use the solution in step 2.
Other	Less common interferences such as **hydrazine** and **glycine** will cause intensified colors in the prepared sample. **Turbidity** and **color** will give erroneous high values. Samples with severe interferences require distillation. Use the distillation procedure with the General Purpose Distillation Set.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
- If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate* for each 0.3 mg/L Cl_2 in a one-liter sample.
- Preserve samples by reducing the pH to 2 or less with at least 2 mL of Hydrochloric Acid.
- Store at 4 °C (39 °F) or less.
- Preserved samples may be stored up to 28 days.
- Warm stored samples to room temperature before analysis.
- Neutralize preserved samples to a pH of 7.0 with 5.0 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Nitrogen, Ammonia Ampule Standard, 150-mg/L NH_3–N
- Ampule breaker
- TenSette Pipet and Pipet tips
- Mixing cylinders, 25-mL, (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume, and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL, and 0.6 mL of standard to three 25-mL portions of fresh sample in three mixing cylinders.
6. Follow the *Salicylate method, TNT* test procedure for each of the spiked samples, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 100-mg/L Ammonia Nitrogen standard
- Deionized water
- 50-mL Class A volumetric flask
- 20-mL Class A volumetric pipet and pipet filler

1. Prepare a 40.0 mg/L ammonia nitrogen standard solution as follows:
   a. Pipet 20.00 mL of 100-mg/L Ammonia Nitrogen standard, into a 50-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
2. Use the 40.0 mg/L solution in place of the sample. Follow the *Salicylate method, TNT* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
343	40.00 mg/L NH_3–N	38.1–41.9 mg/L NH_3–N	0.312 mg/L NH_3–N

Summary of method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a green-colored solution. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Reagent Set, High Range Test 'N Tube™ AmVer™ Nitrogen Ammonia	2	25 tests	2606945

Required apparatus

Description	Quantity	Unit	Catalog number
Funnel, micro (for adding reagent)	1	each	2584335
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette® Pipet 1970001	varies	50/pkg	2185696
Tube Rack, 16 mm	1	each	1864100

Recommended standards

Description	Unit	Catalog number
Nitrogen Ammonia Standard Solution, 10-mg/L NH_3–N	500 mL	15349
Nitrogen Ammonia Standard Solution, 100-mg/L NH_3–N	500 mL	2406549
Nitrogen Ammonia Standard Solution, 150-mg/L NH_3–N, 10-mL Voulette® Ampules	16/pkg	2128410
Nitrogen Ammonia Standard Solution, 50-mg/L NH_3–N, 10-mL Voluette Ampules	16/pkg	1479110
Wastewater, Effluent Inorganics Standard, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinders, mixing, 25 mL tall form	each	2088640
Distillation Set, general purpose	–	2265300
Filter Paper, folded, 12.5 cm	100/pkg	69257

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet Tips, for TenSette Pipet 19700-01	1000/pkg	2185628
Ampule Breaker, Voluette	each	2196800
Heater and Support Apparatus, 115 VAC, 60 Hz	each	2274400
Heater and Support Apparatus, 230 VAC, 50 Hz;	each	2274402
Filter Funnel, poly, 65 mm	each	108367
Pipet, 2 mL serological	each	50549
Pipet Filler, safety bulb	each	1465100
Hydrochloric Acid Standard Solution, 1 N	500 mL	13449
Sodium Hydroxide Standard Solution, 1 N	100 mL	104532
Sulfide Inhibitor Reagent Powder Pillows	100/pkg	241899
Hydrochloric Acid, conc	500 mL	13449
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	245026

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Edition 7

Nitrogen, Ammonia

DOC316.53.01080

Salicylate Method[1] **Method 10023**

LR (0.02 to 2.50 mg/L NH_3–N) **Test ‘N Tube™ Vials**

Scope and Application: For water, wastewater and seawater

[1] Adapted from *Clin. Chim. Acta*, 14, 403 (1966)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 240 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in cell compartment #2 before performing this test.
Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.
Dispose of reacted solutions according to local, state and federal regulations. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.

Collect the following items:

Description	Quantity
Low Range Test ‘N Tube AmVer™ Nitrogen Ammonia Reagent	2
Light Shield (see *Instrument-specific information*)	1
Funnel, micro (for adding reagent)	1
Pipet, TenSette®, 1.0–10.0 mL	1
Pipet Tips, for TenSette Pipet	varies

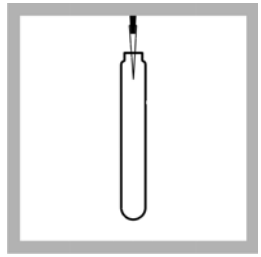
See *Consumables and replacement items* for reorder information.

Salicylate method, LR, TNT

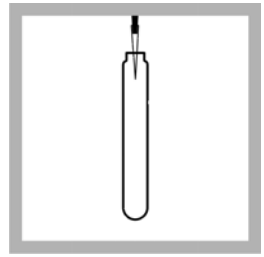
1. Select the test.

Insert an adapter or light shield if required (see *Instrument-specific information*).

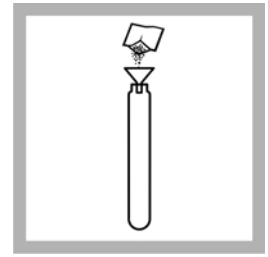
Refer to the user manual for orientation.



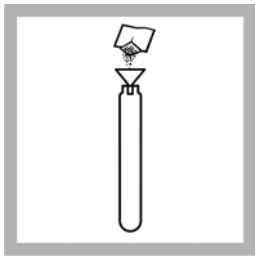
2. Prepared Sample: Add 2.0 mL of sample to one AmVer™ Diluent Reagent Test 'N Tube for Low Range Ammonia Nitrogen.



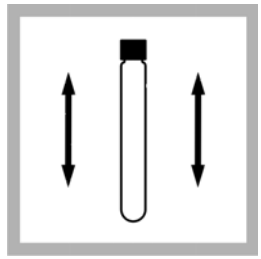
3. Blank Preparation: Add 2.0 mL of ammonia-free water to one AmVer™ Diluent Reagent Test 'N Tube for Low Range Ammonia Nitrogen.



4. Add the contents of one Ammonia Salicylate Reagent Powder Pillow to each vial.



5. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each vial.

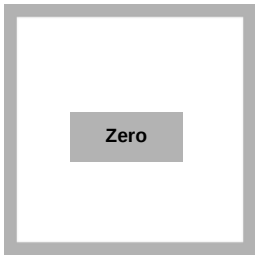


6. Cap the vials tightly and shake thoroughly to dissolve the powder.

7. Start the instrument timer.

A 20-minute reaction period will begin.

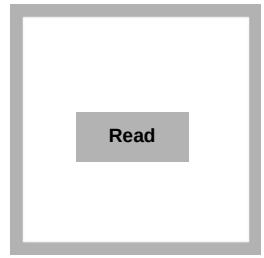
8.



9. **ZERO** the instrument.

The display will show: 0.00 mg/L NH_3–N

10.



11. **READ** the results in mg/L NH_3–N.

Interferences

Table 241 Interfering substances

Interfering substance	Interference level
Calcium	2500 mg/L as $CaCO_3$
Iron	Determine the amount of iron present in the sample by using one of the Iron, Total, procedures. Add the same iron concentration to the ammonia-free water in step 3. The interference will then be successfully blanked out.
Magnesium	15,000 mg/L as $CaCO_3$
Monochloramine	Monochloramine present in chloraminated drinking water Interferes directly at all levels giving high results. Use Method 10200, Free Ammonia and Monochloramine, to determine free ammonia in these sample matrices.
Nitrate	250 mg/L as NO_3^-–N
Nitrite	30 mg/L as NO_2^-–N
Orthophosphate	250 mg/L as PO_4^{3-}–P
pH	Acidic or basic samples should be adjusted to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution[1] for acidic samples and 1 N Hydrochloric Acid Standard Solution[1] for basic samples.
Sulfate	300 mg/L as SO_4^{2-}
Sulfide	**1.** Measure about 350 mL of sample in a 500-mL Erlenmeyer flask. **2.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow[1]. Swirl to mix. **3.** Filter the sample through a folded filter paper[1]. **4.** Use the filtered solution in step 3.
Other	Less common interferences such as **hydrazine** and **glycine** will cause intensified colors in the prepared sample. **Turbidity** and **color** will give erroneous high values. Samples with severe interferences require distillation. Use the distillation procedure with the General Purpose Distillation Set.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
- If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate* for each 0.3 mg/L Cl_2 in a one-liter sample.
- Preserve samples by reducing the pH to 2 or less with at least 2 mL of Hydrochloric Acid.
- Store at 4 °C (39 °F) or less.
- Preserved samples may be stored up to 28 days.
- Warm stored samples to room temperature before analysis.
- Neutralize preserved samples to a pH of 7.0 with 5.0 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Nitrogen, Ammonia Ampule Standard, 50-mg/L NH_3–N
- Ampule breaker
- TenSette Pipet and Pipet Tips
- Mixing cylinders, 25-mL, (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample in three mixing cylinders.
6. Follow the *Salicylate method, LR, TNT* test procedure for each of the spiked samples, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 1.0-mg/L Ammonia Nitrogen standard

1. Use a 1.0-mg/L Ammonia Nitrogen standard in place of the sample. Follow the *Salicylate method, LR, TNT* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**. .
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—DConcentration per 0.010 DAbs
342	1.00 mg/L NH_3–N	0.90–1.10 mg/L NH_3–N	0.014 mg/L NH_3–N

Summary of method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a green-colored solution. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Reagent Set, Low Range Test 'N Tube™ AmVer™ Nitrogen Ammonia	2	25 tests	2604545

Required apparatus

Description	Quantity	Unit	Catalog number
Funnel, micro (for adding reagent)	1	each	2584335
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Tube Rack, 16 mm	1–3	each	1864100

Recommended standards

Description	Unit	Catalog number
Nitrogen Ammonia Standard Solution, 1.0-mg/L NH_3–N	500 mL	189149
Nitrogen Ammonia Standard Solution, 50-mg/L NH_3–N, 10-mL Voluette® Ampules	16/pkg	1479110
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
Wastewater, Effluent Inorganics Standard, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Water, deionized	4L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule Breaker, Voluette	each	2196800
Cylinders, mixing, 25 mL	each	2088640
Distillation Set, general purpose	each	2265300
Filter Funnel, poly, 65 mm	each	108367
Filter Paper, folded, 12.5 cm	100/pkg	189457
Heater and Support Apparatus, 115 VAC, 60 Hz	each	2274400
Heater and Support Apparatus, 230 VAC, 50 Hz	each	2274402
Hydrochloric Acid Standard Solution, 1 N	1000 mL	2321353
Hydrochloric Acid, concentrated ACS	500 mL	13449
Pipet Tips, for TenSette Pipet 1970010	50/pkg	2199796
Pipet, 2 mL serological	each	50549
Pipet Filler, safety bulb	each	1465100
Sodium Hydroxide Standard Solution, 1 N	100 mL	104532

Optional reagents and apparatus

Description	Unit	Catalog number
Sodium Hydroxide Standard Solution, 5.0 N	100 mL	245026
Sulfide Inhibitor Reagent Powder Pillows	100/pkg	241899

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Edition 7

Nitrogen, Free Ammonia

DOC316.53.01084

Indophenol Method[1] **Method 10201**

(0.01 to 0.50 mg/L NH_3–N) **Powder Pillows**

Scope and Application: For controlling free ammonia levels during the production of chloramines, at booster stations and for monitoring free ammonia levels in potable distribution system waters.

[1] U.S. Patent 6,315,950

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 242 Instrument-specific information

Instrument	Sample Cell	Cell Orientation	Adapter
DR 6000	4864302	Orientation key faces right	—
DR 5000	4864302	Orientation key toward user	A23618
DR 3900	4864302	Orientation key inserted in adapter slot	LZV846 (A)
DR 3800, DR 2800, DR 2700	5940506	1-cm (flat) path aligned with the arrow on the adapter	LZV585 (B)

Before starting the test:

Use Method 10200, Nitrogen, Free Ammonia and Chloramine (Mono) to determine free ammonia and monochloramine simultaneously on the same sample.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.
Dispose of reacted solutions according to local, state and federal regulations. Use the guidance given on the Material Safety Data Sheets to dispose of unreacted reagents. Consult local regulatory agencies for further disposal information.

Description	Quantity
Free Ammonia Reagent Set	—
Free Ammonia Reagent Solution	1 drop
Monochlor F Reagent Pillows	2
Sample Cell (see *Instrument-specific information*)	2

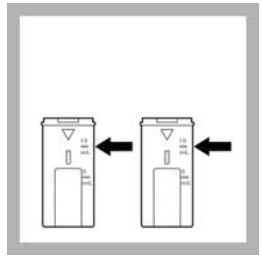
See *Consumables and replacement items* for reorder information.

Indophenol method for powder pillows

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Fill two cells to the 10-mL line with sample.

Label one cell ‘sample’ and one cell ‘blank’.

3. Prepared Sample: Add one drop of Free Ammonia Reagent Solution to the sample.

4. Cap the reagent bottle to maintain reagent performance and stability.

5. Cap and invert the sample to mix.

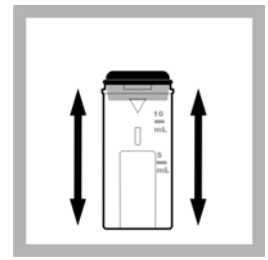
If the sample becomes cloudy by the end of the reaction period, pretreat the sample and retest. See *Interferences*.

6. Start the instrument timer.

A 5-minute reaction period will begin.

Color development time depends on sample temperature. For accurate results allow the full reaction period to occur. See *Color development based on sample temperature*.

7. When the timer expires, add the contents of one MonoChlor F powder pillow to each cell.



8. Cap and shake both cells about 20 seconds to dissolve the reagent.

A green color will develop if monochloramine is present.

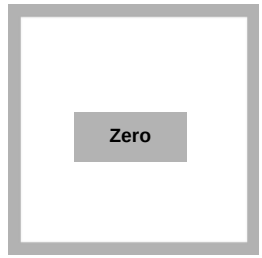
Indophenol method for powder pillows (continued)

9. Start the instrument timer.

A 5-minute reaction period will begin.

Color development time depends on sample temperature. For accurate results allow the full reaction period to occur. See *Color development based on sample temperature*.

10. When the timer expires, insert the blank vial into the cell



11. **ZERO** the instrument.

The display will show: 0.00 mg/L NH_3–N f

Remove the blank.

12. Insert the sample into the cell

13. **READ** the results in mg/L NH_3–N f.

Interferences

This method is intended for finished, chloraminated drinking water samples that have a measurable combined (total) chlorine disinfectant residual. Samples where the disinfectant residual has disappeared and samples which exhibit a chlorine demand may produce low ammonia test results. Blanks and ammonia standards analyzed without a disinfectant residual must be prepared using high quality, reagent grade water.

The following do not interfere in the free ammonia determination at or below the stated concentration.

Table 243 Interfering substances

Interfering substance	Interference level
Aluminum	0.2 mg/L
Chloride	1200 mg/L Cl^-
Copper	1 mg/L Cu
Iron	0.3 mg/L Fe
Manganese	0.05 mg/L Mn
Nitrate	10 mg/L NO_3–N
Nitrite	1 mg/L NO_2–N
Phosphate	2 mg/L o–PO_4
Silica	100 mg/L SiO_2
Sulfate	1600 ppm as $CaCO_3$
Zinc	5 ppm Zn

Samples containing high levels of both Total Hardness and Alkalinity may become cloudy after the addition of the Free Ammonia Reagent Solution. If this occurs by the end of the first reaction period, the sample for Free Ammonia measurement must be pretreated as follows:

Note: *The blank does not need pretreatment.*

1. Measure 10 mL of sample into the cell labeled for the sample.
2. Add the contents of one Hardness Treatment Reagent Powder Pillow to the sample.
3. Cap the cell and invert until the reagent is dissolved.
4. Remove the cap.
5. Continue with the analysis at step 2 using the pretreated sample as the sample.

Color development time

Test results are strongly influenced by sample temperature. **Both reaction periods in the procedure are the same and depend on the temperature of the sample.** The reaction periods indicated in the procedure are for a sample temperature of 18–20 °C (64–68 °F). Adjust both reaction periods (see *Color development based on sample temperature*). The samples can be read up to 15 minutes after the development times listed in Table 244 as the developed color is stable for an extended period of time.

Table 244 Color development based on sample temperature

Sample Temperature		Development Time (minutes)
°C	°F	
5	41	10
7	45	9
9	47	8
10	50	8
12	54	7
14	57	7
16	61	6
18	64	5

Table 244 Color development based on sample temperature

Sample Temperature		Development Time (minutes)
°C	°F	
20	68	5
23	73	2.5
25	77	2
greater than 25	greater than 77	2

Sample collection, preservation and storage

Collect samples in clean glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.

Accuracy check

Dilution water is required when testing a diluted sample and preparing standard solutions. Dilution water must be free of ammonia, chlorine and chlorine demand. A convenient source is a recirculating, deionizer system with carbon filtration which produces 18 megohm-cm water.

Standard additions method (sample spike)
Required for accuracy check:

- Ammonia Nitrogen Standard, 10 mg/L as NH_3-N
- Ampule breaker
- TenSette Pipet and Pipet Tips
- Mixing cylinders, 50 mL (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution.
5. Use the TenSette Pipet to prepare spiked samples: add 0.3 mL, 0.6 mL and 1.0 mL of standard to three 50-mL portions of fresh sample.
6. Follow the *Indophenol method for powder pillows* test procedure for each of the spiked samples, starting with the 0.3 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Ammonia Nitrogen Standard Solution, 10 mg/L NH_3–N
- Deionized water
- Volumetric Pipet, 2 mL or Tensette Pipet and Pipet tips
- Volumetric flask, 100 mL

1. Prepare a 0.20 mg/L ammonia nitrogen standard solution as follows:
   - Dilute 2.00 mL of Ammonia Nitrogen Standard Solution, 10 mg/L to 100 mL with deionized water.
   - Or, use the TenSette Pipet to dilute 0.4 mL of a Ammonia Nitrogen Voluette Standard Solution, 50 mg/L as NH_3–N, to 100 mL with dilution water.
2. Use this solution in place of the sample. Follow the *Indophenol method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
388	0.20mg/L NH_3–N	0.19–0.21 mg/L NH_3–N	0.01 NH_3–N

Summary of method

Monochloramine (NH_2Cl) and free ammonia (NH_3 and NH_4^+) can exist in the same water sample. Added hypochlorite combines with free ammonia to form more monochloramine. In the presence of a cyanoferrate catalyst, monochloramine in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample. Free ammonia is determined by comparing the color intensities, with and without added hypochlorite. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Free Ammonia Reagent Set (50 tests), includes:	—	—	2879700
(1) 2802299, (1) 2877336			
Free Ammonia Reagent Solution	1 drop	4 mL SCDB	2877336
Monochlor F Reagent Pillows	2	100/pkg	2802299

Recommended standards

Description	Unit	Catalog number
Hardness Treatment Reagent Pillows (1 per test)	50/pkg	2882346
Nitrogen Ammonia Standard Solution, 10 mg/L as NH_3–N	500 mL	15349
Nitrogen Ammonia Standard Ampule, 50 mg/L as NH_3–N, 10 mL	16/pkg	1479110
Water, organic-free water	500-mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
Ampule Breaker Kit	each	2196800
Flask, volumetric, Class A, 100 mL	each	1457442
Pipet Filler, Safety Bulb	each	1465100
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Thermometer, –10 to 110 °C	each	187701
Wipers, Disposable, 30 x 30 cm, 280/box	box	2097000
Pipet, volumetric, Class A, 2.0 mL	each	1451536
Cylinder, graduated mixing, 50 mL	each	189641

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Edition 7

Nitrogen, Total

DOC316.53.01085

Persulfate Digestion Method **Method 10072**

HR (2 to 150 mg/L N) **Test 'N Tube Vials**

Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 245 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800, DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
Digestion is required for determining total nitrogen.
This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.
If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.
Use the deionized water provided in the reagent set or Organic-free Water to prepare the standards and perform the procedure.

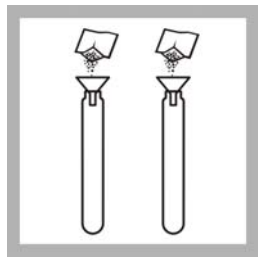
Collect the following items:

Description	Quantity
Test ‘N Tube™ HR Total Nitrogen Reagent Set	1
DRB200 Reactor	1
Funnel, micro	1
Light Shield or Adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 0.1 to 1.0 mL plus tips	1
Pipet, TenSette®, 1.0 to 10.0 mL plus tips	1
Test Tube Cooling Rack	1

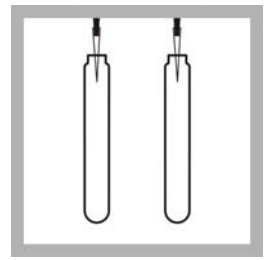
See *Consumables and replacement items* for reorder information.

Persulfate digestion method

1. Turn on the DRB200 Reactor and heat to 105 °C.

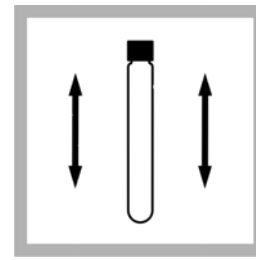


2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two HR Total Nitrogen Hydroxide Digestion Reagent vials. Wipe off any reagent that may get on the lip or the tube threads.



3. Prepared Sample: Add 0.5 mL of sample to a vial.

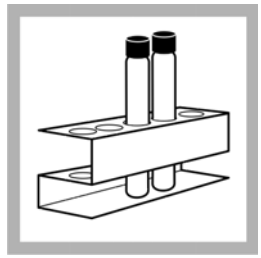
Blank Preparation: Add 0.5 mL of the deionized water included in the kit to a second vial. Use only water that is free of all nitrogen-containing species as a substitute for the deionized water provided.



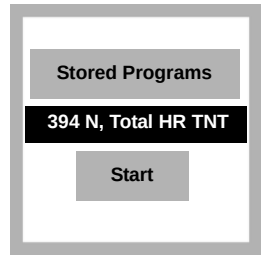
4. Cap both vials. Shake vigorously for at least 30 seconds to mix. The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.

Persulfate digestion method (continued)

5. Insert the vials in the reactor and close the lid. Heat for exactly 30 minutes.

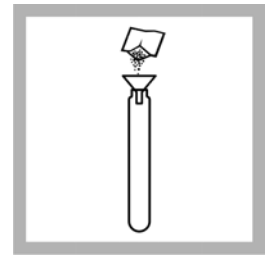


6. Using finger cots, immediately remove the hot vials from the reactor. Cool the vials to room temperature.

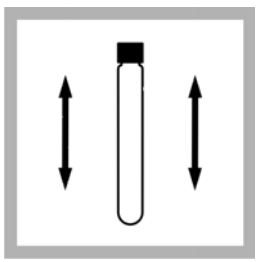


7. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



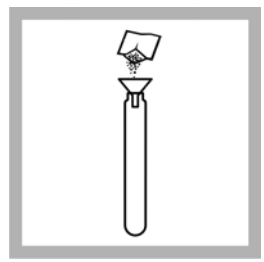
8. Remove the caps from the digested vials and add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.



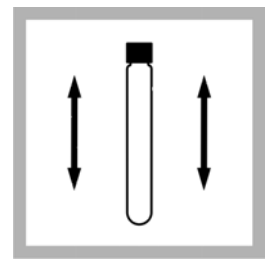
9. Cap the tubes and shake for 15 seconds.

10. Start the instrument timer.

A three-minute reaction period will begin.



11. After the timer expires, remove the caps from the vials and add one TN Reagent B Powder Pillow to each vial.



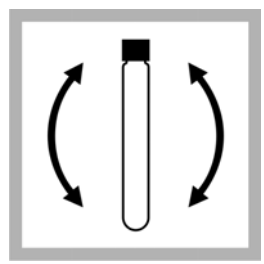
12. Cap the tubes and shake for 15 seconds. The reagent will not completely dissolve. This will not affect accuracy. The solution will begin to turn yellow.

13. Start the instrument timer.

A two-minute reaction period will begin.

14. Prepared Sample: After the timer expires, pipet 2 mL of digested, treated sample into one TN Reagent C vial.

Blank: Add 2 mL of digested, treated reagent blank to the second TN Reagent C vial.



15. Cap the vials and invert ten times to mix. Use slow, deliberate inversions for complete recovery.

The tubes will be warm to the touch.

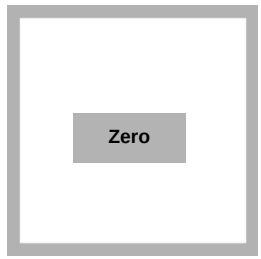
16. Start the instrument timer.

A five-minute reaction period will begin.

The yellow color will intensify.

Persulfate digestion method (continued)

17.

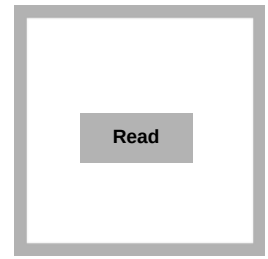


18. **ZERO** the instrument.

The display will show:

0 mg/L N

19. Wipe the reagent vial and insert it into the 16-mm round cell holder.



20. **READ** the results in mg/L N.

Blanks for colorimetric measurement

The reagent blank may be used up to seven days for measurements using the same lots of reagents. Store it in the dark at room temperature (18–25 °C). If a small amount of white floc appears within a week, discard the reagent blank and prepare a new one.

Interferences

The substances in the *Non-interfering substances* table have been tested and found not to interfere up to the indicated levels (in mg/L). Interfering substances that resulted in a concentration change of ±10% appear in the *Interfering substances* table.

Table 246 Non-interfering substances

Interfering substance	Interference level
Barium	10.4 mg/L
Calcium	1200 mg/L
Chromium ($^{3+}$)	2 mg/L
Iron	8 mg/L
Lead	26.4 µg/L
Magnesium	2000 mg/L
Organic Carbon	600 mg/L
Phosphorus	400 mg/L
Silica	600 mg/L
Silver	3.6 mg/L
Tin	6 mg/L

Table 247 Interfering substances

Interfering substance	Interference level
Bromide	> 240 mg/L; positive interference
Chloride	> 3000 mg/L; positive interference

This test performed with standard nitrogen solutions prepared from the following compounds obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in ≥ 95% recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
- Preserve the sample by reducing the pH to 2 or less with concentrated (at least 2 mL/L) Sulfuric Acid.
- Store samples at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days.
- Warm stored samples to room temperature and neutralize with 5 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy use Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following three solutions. Each preparation is for an equivalent 120-mg/L N standard. Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.
   - Weigh 1.6208 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   - Weigh 2.1179 g of Glycine p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   - Weigh 2.5295 g of Nicotinic p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula. Refer to the *Percent recovery* table for more information.

$$\%\ \text{recovery} = \frac{\text{measured concentration}}{120} \times 100$$

Table 248 Percent recovery

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard additions method (sample spike)
Required for accuracy check:

- Ammonia Nitrogen Standard Solution, 1000-mg/L as NH_3–N
- TenSette Pipet and Pipet tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
5. Follow the *Persulfate digestion method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
6. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 100-mg/L ammonia nitrogen standard solution

1. Substitute 0.5 mL of a 100-mg/L ammonia nitrogen standard solution in place of the sample. Follow the *Persulfate digestion method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**..
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
394	100 mg/L NH_3–N	98–102 mg/L N	0.5 mg/L N

Summary of method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Test 'N Tube™ Total Nitrogen Reagent Set	50 vials	2714100

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	2	50/pkg	2185696
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	2	50/pkg	2199796
Test Tube Cooling Rack	1	each	1864100
Finger cots	2	2/pkg	1464702

Recommended standards

Description	Unit	Catalog number
Ammonia Nitrogen Standard Sol., 1000-mg/L NH_3–N	1 L	2354153
Ammonia Nitrogen Standard Sol., 100-mg/L NH_3–N	500 mL	2406549
Analytical balance, 80 g capacity, 0.1 mg resolution	each	2936701
Cylinder, mixing with stopper, 25 mL	each	2088640
Flask, volumetric, Class A, 1000 mL	each	1457453
Pipet tips for 1970001	1000/pkg	2185628
Pipet tips for 1970010	250-pkg	2199725
Primary Standard Set, for Kjeldahl Nitrogen	set of 3	2277800
Sodium Hydroxide, 5 N	50 mL	245026
Sulfuric Acid, ACS	500 mL	97949
Wastewater Mixed Inorganic Standard for NH_3-H, NO_3-N, PO4, COD, SO_4,TOC	500 mL	2833149
Water, deionized	500 mL	27249
Water, organic-free	500 mL	2641549
Weighing papers	500/pkg	1473800
PourRite Ampule breaker 2-mL	each	2484600
Voluette Ampule breaker 10 mL	each	2196800
Ammonia Nitrogen Standard Solution, 10-mL Voluette® Ampules, 50 mg/L	16/pkg	1479110
Ammonia Nitrogen Standard Solution, 10-mL Voluette® Ampules, 150 mg/L	16/pkg	2128410

Recommended standards

Description	Unit	Catalog number
Ammonia Nitrogen Standard Solution, 2-mL PourRite® Ampules, 50 mg/L	20/pkg	1479120
Ammonia Nitrogen Standard Solution, 10-mg/L NH_3–N	500 mL	15349

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Edition 7

Nitrogen, Total Inorganic

DOC316.53.01090

Titanium Trichloride Reduction Method **Method 10021**

0.2 to 25.0 mg/L N **Test ‘N Tube™ Vials**

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 249 Instrument-specific information

Instrument	Sample cell
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

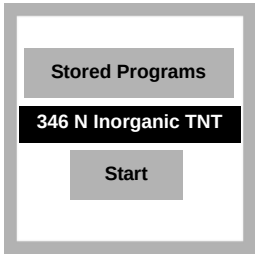
DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in cell compartment #2 before performing this test.
For safety, wear gloves while breaking ampules.
The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. Refer to the current MSDS for safe handling and disposal information.

Collect the following items:

Description	Quantity
Total Inorganic Nitrogen Pretreatment Reagent Set ($TiCl_3$ Reduction Method)	1
Test ‘N Tube™ AmVer™ Nitrogen-Ammonia Reagent Set	1
Water, deionized	1 mL
Centrifuge	1
Funnel, micro	1
Light Shield or adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 1.0–10.0 mL with tips	1
Pipette, volumetric, Class A, 1.00-mL	1
Test Tube Rack	1

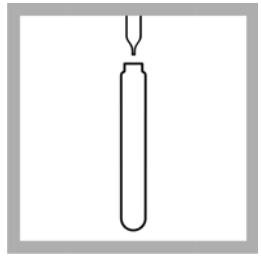
See *Consumables and replacement items* for reorder information.

Titanium Trichloride Reduction method, TNT

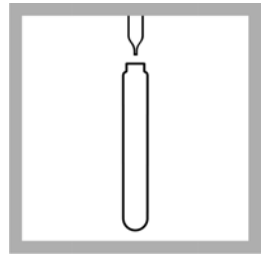


1. Select the test.

Insert an adapter or light shield if required (see *Instrument-specific information*).

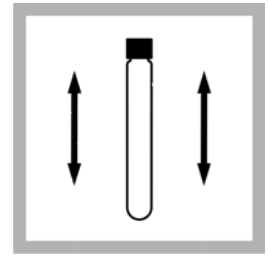


2. Pipet 1 mL of Total Inorganic Nitrogen Pretreatment Base concentrate into each of two Total Inorganic Nitrogen Pretreatment Diluent Vials.

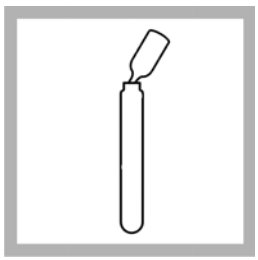


3. Prepared Sample: Pipet 1 mL of sample into one vial.

Blank Preparation: Pipet 1 mL of deionized water into the second vial.



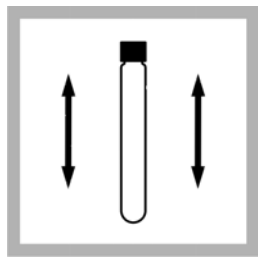
4. Cap the vials and shake for 30 seconds to mix.



5. Pour the contents of one Total Inorganic Nitrogen Reductant ampule into the vial containing the sample.

Pour the contents of another Total Inorganic Nitrogen Reductant ampule into the vial containing the blank.

A black precipitate will form immediately.



6. Immediately cap the vials. Shake gently for 30 seconds to mix the reagents. Allow the vials to stand for at least one minute.

The precipitate should remain black after shaking. Excessive shaking will cause the precipitate to turn white and cause low results.

7. Insert the vials in a centrifuge.

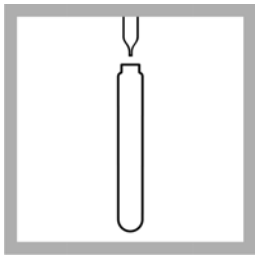
(If a centrifuge is not available, wait at least 30 minutes for the solids to settle at the bottom of the vial. Proceed to step 9.)

8. Start the instrument timer.

A three-minute timer will begin.

Centrifuge the vials for three minutes.

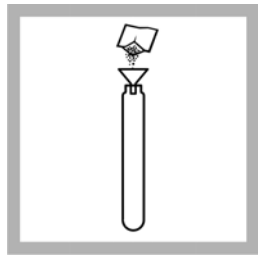
Titanium Trichloride Reduction method, TNT (continued)



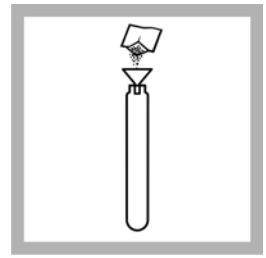
9. Using a pipet, add 2 mL of centrifuged sample to an AmVer™ Diluent Reagent Test 'N Tube™ for Low Range Ammonia Nitrogen.

Add 2 mL of centrifuged blank to another AmVer™ Diluent Reagent Test 'N Tube™ for Low Range Ammonia Nitrogen.

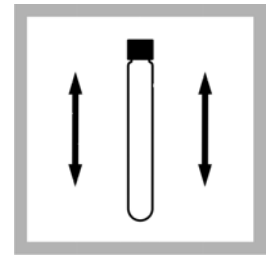
Pipet carefully to avoid disturbing the sediment.



10. Using a funnel, add the contents of one Ammonia Salicylate Reagent Powder Pillow (for 5-mL samples) to each vial.



11. Using a funnel, add the contents of one Ammonia Cyanurate Reagent Powder Pillow (for 5-mL samples) to each vial.



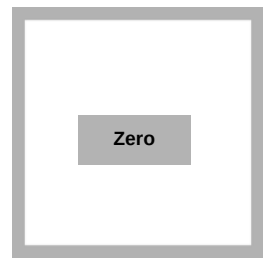
12. Cap the vials tightly and shake thoroughly to dissolve the powder.

A green color will develop if nitrogen is present.

13. Start the instrument timer.

A 20-minute reaction period will begin.

14.



15. **ZERO** the instrument.

The display will show:

0.0 mg/L N

16. Wipe the prepared sample and insert it into the 16-mm round cell holder.

READ the results in mg/L N.

Interferences

The substances in the *Interfering substances* table may interfere when present. The substances in the *Non-interfering substances* table do not interfere below the levels listed.

Table 250 Interfering substances

Interfering substance	Interference level
Calcium	Causes a positive interference at 1000 mg/L as $CaCO_3$.
Manganese (IV)	Causes a negative interference at 3 mg/L.
Magnesium	Causes a positive interference at 1000 mg/L as $CaCO_3$.
Sulfide	Causes a negative interference at 3 mg/L.
Sulfate	Causes a negative interference at 250 mg/L.

Table 251 Non-interfering substances

Interfering substance	Interference level
Al^{3+}	8 mg/L
Ba^{2+}	40 mg/L
Cu^{2+}	40 mg/L
Fe^{3+}	8 mg/L
Zn^{2+}	80 mg/L
F^-	40 mg/L
PO_4^{3-}–P	8 mg/L
SiO_2	80 mg/L
EDTA	80 mg/L

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
- If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate* for each 0.3 mg/L Cl_2 in a one-liter sample.
- Preserve samples by reducing the pH to 2 or less with concentrated (at least 2 mL) Hydrochloric Acid*. Store at 4 °C (39 °F) or less.
- Preserved samples may be stored up to 28 days.
- Before analysis, warm stored samples to room temperature and neutralize with 5 N Sodium Hydroxide*.
- Correct the test result for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- HR Nitrate Nitrogen PourRite Ampule Standard, 500-mg/L NO_3^-–N
- Ampule breaker
- TenSette Pipet 0.1–1.0 mL and tips
- 25 mL Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
6. Follow the *Titanium Trichloride Reduction method, TNT* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 10.0-mg/L Nitrate Nitrogen Standard Solution

1. Use this solution in place of the sample. Follow the *Titanium Trichloride Reduction method, TNT* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

The total inorganic nitrogen test is designed to provide an estimate of the total nitrite, nitrate and ammonia nitrogen load present in a water or wastewater sample. This test is most applicable to the monitoring of samples taken from an industrial process stream or a wastewater treatment stream where it is important to track the inorganic nitrogen load as it passes through the treatment process. The test does exhibit different recoveries of each of the three nitrogen species, as summarized below. The test is not recommended for use when quantifying only one of the three species. In that case, specific procedures for each particular analyte would be more appropriate.

Species Recovery

Nitrogen Form	Recovery
NH_3–N	112%
NO_3^-–N	100%
NO_2^-–N	77%

Program	Standard	95% Confidence Limits of Distribution	Sensitivity—DConcentration per 0.010 DAbs
346	20.0 mg/L NO_3^-–N	19.6–20.4 mg/L NO_3^-–N	0.2 mg/L NO_3^-–N

Summary of method

Titanium (III) ions reduce nitrate and nitrite to ammonia in a basic environment. After centrifugation to remove solids, the ammonia is combined with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green colored solution. Test results are measured at 655 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total Inorganic Nitrogen Pretreatment Reagent Set ($TiCl_3$ Reduction Method)	—	25 tests	2604945
Test 'N Tube™ AmVer™ Nitrogen-Ammonia Reagent Set	—	25 tests	2604545
Water, deionized	1 mL	100 mL	27242

Required apparatus

Description	Quantity	Unit	Catalog number
Centrifuge, 115 VAC, 6 x 15 mL	1	each	2676500
OR			
Centrifuge, 220 VAC, 6 x 15 mL	1	each	2676502
Funnel, micro	1	each	2584335
Pipet, TenSette®, 1.0–10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	varies	50/pkg	2199796
Test Tube Rack	1	each	1864100
Gloves, Nitrile, Large[1]	1 pair	100/pkg	2550503

[1] Other sizes are available

Recommended standards

Description	Unit	Catalog number
Flask, volumetric Class A, 50-mL	each	1457441
Nitrate Nitrogen Standard Solution, 10-mg/L NO_3^-–N	500 mL	30749
Nitrate Nitrogen Standard Solution, 2-mL PourRite® Ampule, 500 mg/L	20/pkg	1426020
Pipet Filler, safety bulb	each	1465100
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	2185696
Pipet Tips, for TenSette Pipet 19700-01	1000/pkg	2185628
Pipet Tips, for TenSette Pipet 19700-10	250-pkg	2199725
Water, deionized	4 liters	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, 25 mL mixing, tall form	each	2088640
Hydrochloric Acid, concentrated	500 mL	13449
Sodium Hydroxide, 5.0 N	50 mL SCDB	245026
Sodium Thiosulfate, 0.1 N	100 mL	32332
Pipette, volumetric, Class A, 1.00-mL	each	1451535
PourRite Ampule breaker 2-mL	each	2484600
Voluette Ampule breaker 10 mL	each	2196800
Nitrate Nitrogen Standard Solution, 1-mg/L NH_3–N	500 mL	204649
Nitrate Nitrogen Standard Solution, 100-mg/L NH_3–N	500 mL	194749
Nitrate Nitrogen Standard Solution, 1000-mg/L NH_3–N	500 mL	1279249
Nitrate Nitrogen Standard Solution, MDB, 15-mg/L NH_3–N	100 mL	2415132

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Edition 7

Nitrogen, Total Kjeldahl

DOC316.53.01091

Nessler Method[1] (Digestion Required)

Method 8075

1 to 150 mg/L

Scope and Application: For water, wastewater and sludge; digestion is required.

[1] Adapted from Hach, et. al., *Journal of Association of Official Analytical Chemists*, 70(5) 783-787 (1987); Hach, et. al., *Journal of Agricultural and Food Chemistry*, 33(6) 1117-1123 (1985); *Standard Methods for the Examination of Water and Wastewater*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 252 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

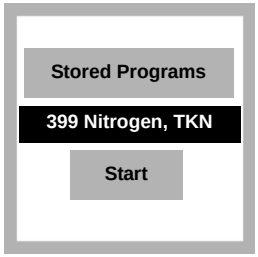
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
If using a Pour-Thru system, periodically clean the cell by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel or rinse with a solution of sodium thiosulfate. Flush it through the funnel and cell with enough deionized water to dissolve. Rinse out the crystals.
Hold droppers and dropper bottles vertically, **not at an angle**, when dispensing reagent.
Nessler reagent contains mercuric iodide. Both the sample and blank will contain mercury (D009) at concentrations regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. Refer to the current MSDS for safe handling and disposal instructions.
Because of the method's sensitivity, it is recommended to use the Standards and Calibration Adjust feature..

Collect the following items:

Description	Quantity
Boiling Chips, silicon carbide	2–3
Cots, finger	2
Cylinder, graduated mixing, 25 mL	2
Digesdahl Digestion Apparatus	1
Hydrogen Peroxide, 50%	20 mL
Mineral Stabilizer	6 drops
Nessler Reagent	2 mL
Polyvinyl Alcohol Dispersing Agent	6 drops
Potassium Hydroxide (KOH) Standard Solution, 1.0 N	varies
Potassium Hydroxide (KOH) Standard Solution, 8.0 N	varies
Sulfuric Acid, ACS, concentrated	6 mL
TKN Indicator Solution	2 drops
Pipet, TenSette®, 0.1–1.0 mL, plus tips	1
Safety Shield	1
Sample Cell (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

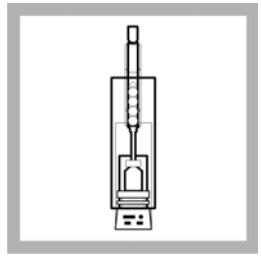
Nessler method



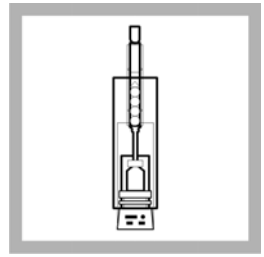
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

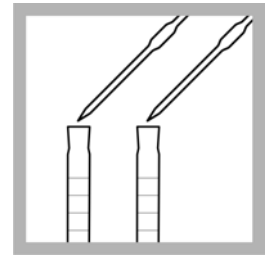
Refer to the user manual for orientation.



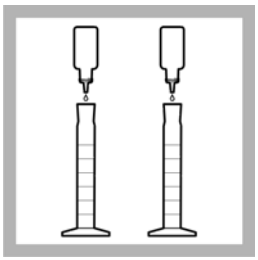
2. Prepared Sample: Digest the sample amount as described in the Digesdahl® Digestion Apparatus Instruction Manual.



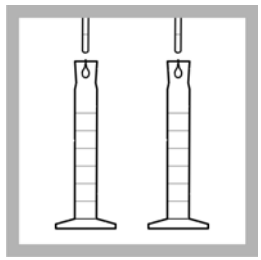
3. Blank Preparation: Digest an equal amount of deionized water as the blank.



4. Select the appropriate analysis volume of the digested sample given in the *Aqueous samples (solutions or suspensions in water—less than 1% solids)*, *Dry samples* or *Oils and fats* tables depending on the sample. Pipet the analysis volume from the sample and the blank into separate 25-mL mixing graduated cylinders.

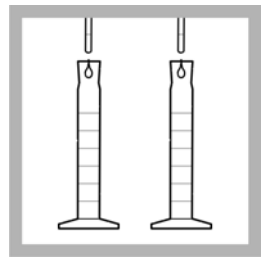


5. Add one drop of TKN Indicator to each cylinder.

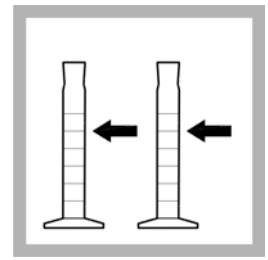


6. If the aliquot is less than 1 mL, proceed to step 7.

If it is greater than 1 mL, add drops of 8.0 N KOH to each cylinder until the first flash of blue color appears. Stopper and invert the cylinder after each addition. Proceed to the next step.

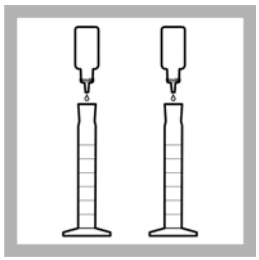


7. Add 1.0 N KOH to each cylinder, one drop at a time, mixing after each addition. Continue until the first permanent blue color appears.

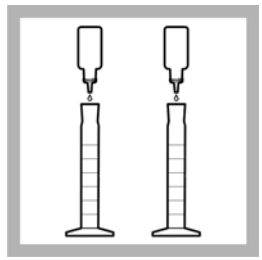


8. Fill both cylinders to the 20-mL mark with deionized water.

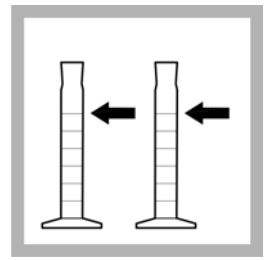
Nessler method (continued)



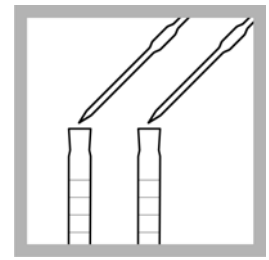
9. Add three drops of Mineral Stabilizer to each cylinder. Stopper and invert several times to mix.



10. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Stopper and invert several times to mix.



11. Fill both cylinders to the 25-mL mark with deionized water. Stopper and invert several times to mix.

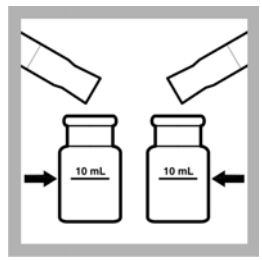


12. Pipet 1.00 mL of Nessler Reagent to each cylinder. Stopper and invert repeatedly.

The solution should not be hazy. Any haze (turbidity) will cause incorrect results.

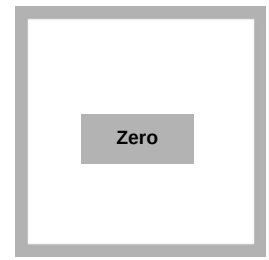
13. Start the instrument timer.

A two-minute reaction period will begin.



14. When the timer expires, pour the contents of the cylinders into separate square sample cells.

15. Wipe the blank and insert it into the cell holder.



16. **ZERO** the instrument.

The display will show:

0 mg/L TKN

Nessler method (continued)

17. Wipe the prepared sample and insert it into the cell holder.

18. **READ** the results in mg/L TKN.

19. Calculate sample TKN as follows:

$$\text{ppm TKN} = \frac{75 \times A}{B \times C}$$

Where:

A = mg/L read from the display

B = g (or mL of water) sample taken for digest

C = mL analysis volume of digested sample

Interferences

Table 253 Aqueous samples (solutions or suspensions in water—less than 1% solids)

Expected nitrogen concentration (mg/L)	Analysis volume (mL)
0.5–28	10.0
2–112	5.0
11–560	2.00
45–2250	1.00
425–22500	0.50

Table 254 Dry samples

Expected nitrogen concentration (mg/L)	Analysis volume (mL)
42–2200	10.0
106–5600	5.00
350–18,000	2.00
1000–56,000	1.00
4200–220,000	0.50

Table 255 Oils and fats

Expected nitrogen concentration (mg/L)	Analysis volume (mL)
85–4500	10.0
210–11,000	5.00
2100–110,000	1.00

Sample collection, preservation and storage

- Collect samples in clean glass or plastic containers.
- Adjust sample pH to 2 or less with Sulfuric Acid (about 2 mL per liter) and cool to 4 °C (39 °F).
- Preserved samples can be stored up to 28 days.

Accuracy check

Kjeldahl Nitrogen standard method
This procedure checks digestion efficiency and indicates the amount of bound nitrogen that is released during digestion. The methods and standards available to check digestion technique are found in the *Accuracy Check* section following the procedure in the *Digesdahl® Digestion Apparatus Instruction Manual*. Using the digested Kjeldahl standard, perform the *Nessler method* on a colorimeter. The TKN value should come within ± 3% of the value of the prepared Kjeldahl standard.

Standard solution method (to check calibration accuracy only)
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 1.0-mg/L NH_3–N solution
- TKN indicator
- Dropper
- Graduated cylinders (2)
- Deionized water
- Mineral Stabilizer
- Polyvinyl Alcohol Dispersing agent

1. Add one drop of TKN Indicator to each 25-mL graduated mixing cylinder.
2. Fill one cylinder to the 20-mL mark with deionized water. Fill the other cylinder to the 20-mL mark with a 1.0-mg/L NH_3–N solution.
3. Add 3 drops of Mineral Stabilizer to each cylinder. Invert several times to mix.
4. Add 3 drops of Polyvinyl Alcohol Dispersing agent to each cylinder. Invert several times to mix.
5. Perform the TKN procedure as described in step 11 to step 18. Accurate calibrations will show 26–27 mg/L TKN.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—DConcentration per 0.010 DAbs
399	76 mg/L NH_3–N	70–82 mg/L NH_3–N	1 mg/L NH_3–N

Summary of method

The term *Total Kjeldahl Nitrogen* refers to the combination of ammonia and organic nitrogen. However, only the organic nitrogen compounds appearing as organically bound nitrogen in the trinegative state are determined in this test. Nitrogen in this form is converted into ammonium salts by the action of sulfuric acid and hydrogen peroxide. The ammonia is then analyzed by a modified Nessler method test. Test results are measured at 460 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Nitrogen Reagent Set, 0–150 mg/L, Nessler Method, includes:	—	250 tests	2495300
Hydrogen Peroxide, 50%	20 mL	490 mL	2119649
Mineral Stabilizer	6 drops	50 mL SCDB	2376626
Nessler Reagent	2 mL	500 mL	2119449
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	2376526
Potassium Hydroxide Standard Solution, 1.0 N	varies	50 mL SCDB	2314426
Potassium Hydroxide Standard Solution, 8.0 N	varies	100 mL MDB	28232H
Sulfuric Acid, ACS, concentrated	6 mL	500 mL	97949
TKN Indicator Solution	2 drops	50 mL SCDB	2251926

Required apparatus

Description	Quantity	Unit	Catalog number
Boiling Chips, silicon carbide	2–3	500 g	2055734
Cots, finger	2	2/pkg	1464702
Cylinder, graduated mixing, 25-mL	2	each	2636240
Digesdahl® Digestion Apparatus, 115 VAC	1	each	2313020
OR			
Digesdahl® Digestion Apparatus, 220 VAC	1	each	2313021
Pipet, TenSette®, 0.1–1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	2	50/pkg	2185696
Safety Shield	1	each	5003000

Recommended standards

Description	Unit	Catalog number
Nitrogen, Primary Standard Set for TKN	each	2277800
Nitrogen Standard Solution, 1-mg/L NH_3–N	500 mL	189149
Nitrogen Standard Solution, Voluette® Ampule, 150-mg/L NH_3–N, 10-mL	16/pkg	2128410
Wastewater Influent Inorganics Standard for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149

Optional reagents and apparatus

Description	Unit	Catalog number
Sodium Thiosulfate, Pentahydrate	454 g	46001
Pour-Thru Cell Kit (DR 2700, DR 2800)	each	5940400
Pour-Thru Cell Kit (DR 5000)	each	LZV479
PourRite Ampule breaker 2-mL	each	2484600
Voluette Ampule breaker 10 mL	each	2196800
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 19700–10	50/pkg	2199796
Pipet Tips, for TenSette Pipet 19700–10	250/pkg	2199725
Pipet Tips, for TenSette Pipet 19700–10	1000/pkg	2185628
Ammonia Nitrogen Standard Solution, 10-mg/L NH_3–N	500 mL	15349
Ammonia Nitrogen Standard Solution, 100-mg/L NH_3–N	500 mL	2406549
Ammonia Nitrogen Standard Solution, 1000-mg/L NH_3–N	1 L	2354153
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 50 mg/L	16/pkg	1479110
Balance, analytical, 80 g capacity	115 VAC	2936701
Weighing Paper, 76 x 76 mm	500/pkg	1473800
Ammonia Nitrogen Standard Solution, 2-mL PourRite® Ampule, 50 mg/L	20/pkg	1479120

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Edition 7

Nitrogen, Total

DOC316.53.001086

Persulfate Digestion Method **Method 10071**

LR (0.5 to 25.0 mg/L N) **Test ‘N Tube™ Vials**

Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 256 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
Digestion is required for determining total nitrogen.
This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.
If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.
Use the deionized water provided in the reagent set or Organic-free Water to prepare the standards and perform the procedure.

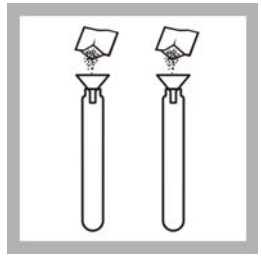
Collect the following items:

Description	Quantity
Test ‘N Tube™ LR Total Nitrogen Reagent Set	1
DRB200 Reactor	1
Funnel, micro	1
Light Shield or adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 1.0 to 10.0 mL plus tips	1
Test Tube Cooling Rack	1–3
Finger Cots	2

See *Consumables and replacement items* for reorder information.

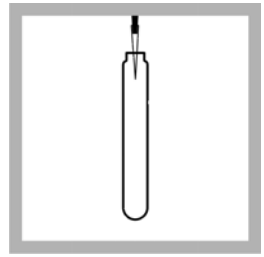
Persulfate digestion method

1. Turn on the DRB200 Reactor and heat to 105 °C.



2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two Total Nitrogen Hydroxide Digestion Reagent vials. Wipe off any reagent that may get on the lip or the tube threads.

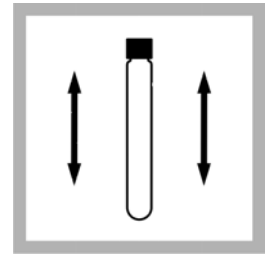
Note: *One reagent blank is sufficient for each set of samples.*



3. Prepared Sample: Add 2 mL of sample to one vial.

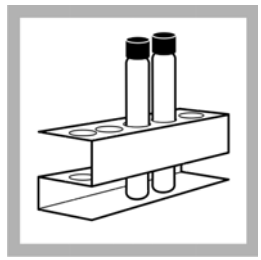
Blank Preparation: Add 2 mL of the deionized water included in the kit to a second vial.

Use only water that is free of all nitrogen-containing species as a substitute for the provided deionized water.

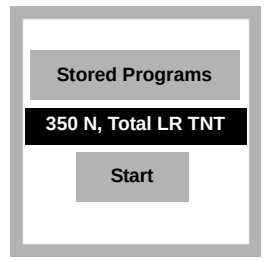


4. Cap both vials. Shake vigorously for at least 30 seconds to mix. The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.

5. Insert the vials in the reactor and close the lid. **Heat for exactly 30 minutes.**

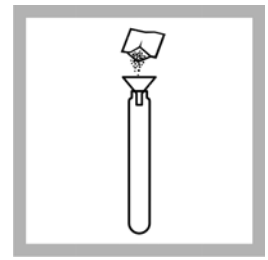


6. Using finger cots, **immediately** remove the hot vials from the reactor. Cool the vials to room temperature.



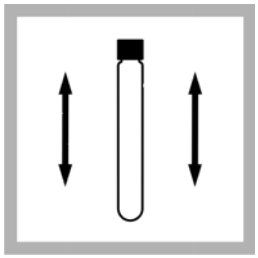
7. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



8. Remove the caps from the digested vials and add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.

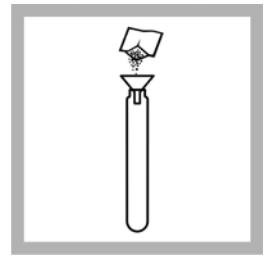
Persulfate digestion method (continued)



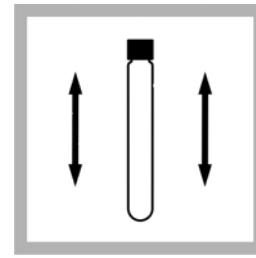
9. Cap the tubes and shake for 15 seconds.

10. Start the instrument timer.

A three-minute reaction period will begin.



11. After the timer expires, remove the caps from the vials and add one TN Reagent B Powder Pillow to each vial.

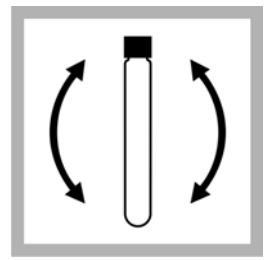


12. Cap the tubes and shake for 15 seconds. The reagent will not completely dissolve. This will not affect accuracy. The solution will begin to turn yellow.

13. Start the instrument timer.

A two-minute reaction period will begin.

14. Prepared Sample: After the timer expires, remove the caps from two TN Reagent C vials and add 2 mL of digested, treated sample to one vial. **Blank:** Add 2 mL of digested, treated reagent blank to the second TN Reagent C vial.



15. Cap the vials and invert ten times to mix. Use slow, deliberate inversions for complete recovery.

The tubes will be warm to the touch.

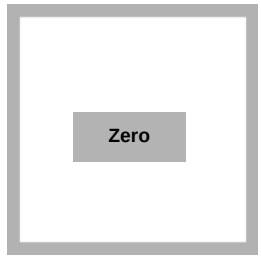
16. Start the instrument timer.

A five-minute reaction period will begin.

The yellow color will intensify.

Persulfate digestion method (continued)

17. Wipe the reagent blank and insert it into the 16-mm round cell holder.



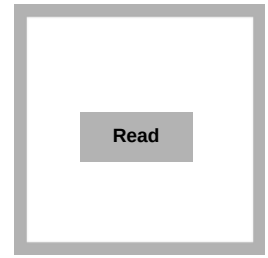
18. **ZERO** the instrument.

The display will show:

0.0 mg/L N

19. Wipe the reagent vial and insert it into the 16-mm round cell holder.

Note: *Multiple samples may be read after zeroing on one reagent blank.*



20. **READ** the results in mg/L N.

Blanks for colorimetric measurement

The reagent blank may be used up to seven days for measurements using the same lots of reagents. Store it in the dark at room temperature (18–25 °C). If a small amount of white floc appears within a week, discard the reagent blank and prepare a new one.

Interferences

The *Non-interfering substances* table shows substances that have been tested and found not to interfere up to the indicated levels (in mg/L). Interfering substances that resulted in a concentration change of ±10% appear in the *Interfering substances* table.

Table 257 Non-interfering substances

Interfering substance	Interference level
Barium	2.6 mg/L
Calcium	300 mg/L
Chromium ($^{3+}$)	0.5 mg/L
Iron	2 mg/L
Lead	6.6 µg/L
Magnesium	500 mg/L
Organic Carbon	150 mg/L
Phosphorus	100 mg/L
Silica	150 mg/L
Silver	0.9 mg/L
Tin	1.5 mg/L

Table 258 Interfering substances

Interfering substance	Interference level
Bromide	> 60 mg/L; positive interference
Chloride	> 1000 mg/L; positive interference

This test performed with standard nitrogen solutions prepared from the following compounds obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in ≥ 95% recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample collection, storage and preservation

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
- Preserve the sample by reducing the pH to 2 or less with concentrated (at least 2 mL/L) Sulfuric Acid.
- Store samples at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days.
- Warm stored samples to room temperature and neutralize with 5 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy use Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following three solutions. Each preparation is for an equivalent 25-mg/L N standard. Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.
   a. Weigh 0.3379 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   b. Weigh 0.4416 g of Glycine p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   c. Weigh 0.5274 g of Nicotinic p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula. Refer to the *Percent recovery* table for more information.

$$\% \text{ recovery} = \frac{\text{measured concentration}}{25} \times 100$$

Refer to the *Percent recovery* table.

Table 259 Percent recovery

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%

Table 259 Percent recovery (continued)

Compound	Lowest Expected % Recovery
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard additions method (sample spike)
Required for accuracy check:

- Ammonia Nitrogen Standard Solution, 1000-mg/L as NH_3–N
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 50-mL portions of fresh sample.
5. Follow the *Persulfate digestion method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
6. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 10-mg/L ammonia nitrogen standard solution

1. Substitute 2 mL of a 10-mg/L ammonia nitrogen standard solution in place of the sample. Follow the *Persulfate digestion method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
350	10 mg/L NH_3–N	9.6–10.4 mg/L N	0.5 mg/L N

Summary of method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Test 'N Tube™ Total Nitrogen Reagent Set, LR	50 vials	2672245

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
DRB200 Reactor, 110 V, 15x16 mm	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 V, 15x16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	2	50/pkg	2199796
Test Tube Cooling Rack	1–3	each	1864100
Finger Cots	2	2/pkg	1464702

Recommended standards

Description	Unit	Catalog number
Ammonia Nitrogen Standard Solution, 1000-mg/L NH_3–N	1 L	2354153
Ammonia Nitrogen Standard Solution, 10-mg/L NH_3–N	500 mL	15349
Primary Standard Set, for Kjeldahl Nitrogen	set of 3	2277800
Wastewater Mixed Inorganic Standard for NH_3-H, NO_3-N, PO4, COD, SO_4,TOC	500 mL	2833149
Water, deionized	500 mL	27249
Water, organic-free	500 mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity, 115 VAC	each	2936701
Cylinder, mixing with stopper, 50 mL	each	2088641
Flask, volumetric, Class A, 1000 mL	each	1457453
Pipet, TenSette, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet tips for TenSette Pipet 1970001	1000/pkg	2185628
Pipet tips for TenSette Pipet 1970010	250-pkg	2199725
Sodium Hydroxide, 5 N	50 mL	245026

Optional reagents and apparatus

Description	Unit	Catalog number
Sulfuric Acid, concentrated	500 mL	97949
PourRite® Ampule breaker, 2-mL	each	2484600
Voluette® Ampule breaker 10 mL	each	2196800
Ammonia Nitrogen Standard Solution, 1-mg/L NH_3–N	500 mL	189149
Ammonia Nitrogen Standard Solution, 100-mg/L NH_3–N	500 mL	2406549
Ammonia Nitrogen Standard Solution, 2-mL PourRite Ampule, 50 mg/L	20/pkg	1479120
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 10 mg/L	16/pkg	1479110
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 150 mg/L	16/pkg	2128410

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Edition 7

Oil and Grease

DOC316.53.01187

USEPA[1] Hexane Extractable Gravimetric Method

Method 10056

15 to 3000 mg/L HEM and SGT-HEM

Scope and Application: For water and wastewater

[1] Equivalent to USEPA Method 1664. Adapted from *Standard Methods for the Examination of Water and Wastewater,* Section 5520B.

Test preparation

Before starting the test:

Determine a blank value (350 mL) of distilled or deionized water) with each new lot of reagents. If the blank result is greater than 5 mg, resolve the source of error or remove interferences before performing this procedure. Use the equivalent amount of acid to determine the blank and all samples from each sampling source.
The sample must be at room temperature before analyzing.
Do not use plastic tubing to transfer the solvent between containers.
Do not pre-rinse the collecting vessel with sample.
Anhydrous sodium sulfate is used to remove traces of water from the hexane extraction layer. Dry the sodium sulfate at 200 to 250 °C for 24 hours for best performance.
Spilled reagent will affect test accuracy and is hazardous to skin and other materials.
If determining both the HEM and the SGT-HEM, clean and dry two distillation flasks (one for each procedure) in advance. • HEM = n-hexane extractable materials • SGT-HEM = Silica Gel Treated n-hexane extractable materials

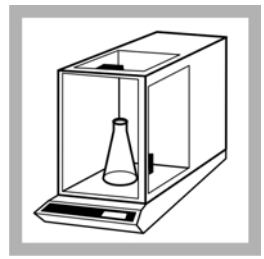
Hexane Extractable Gravimetric Method

1. Collect 350 mL of sample in a clean 500-mL separatory funnel.

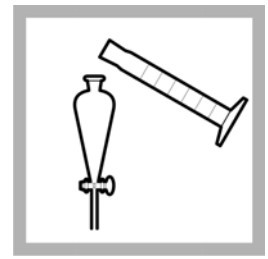
If the sample is not collected in the separatory funnel, set the empty container and lid aside for use in step 4.

2. Using a pipette and pipette filler, add 4 mL of 1:1 Hydrochloric Acid solution to the separatory funnel. Mix well. The pH must be ≤ 2 to hydrolyze oils and grease and prevent a sodium sulfate interference.

Check sample pH after acid addition by dipping a glass rod into the sample and allowing a few drops to touch the pH paper. Do not dip the pH paper into the sample. Rinse the glass rod with a small portion of hexane back into the separatory funnel to remove any grease/oil on the rod.



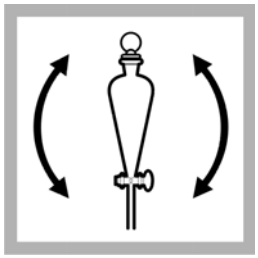
3. Using an analytical balance, weigh a previously dried and cleaned 125-mL distillation flask containing 3–5 boiling chips to the nearest 0.1 mg. Record the weight of the flask.



4. Add 20 mL of n-hexane to the separatory funnel.

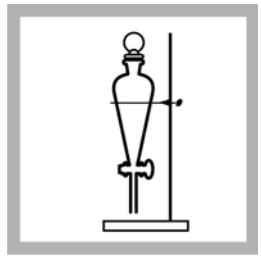
If the sample was collected in a separate container or if repeating this step, rinse the collecting vessel/ volumetric flask which contained the sample/ water layer with 20 mL of n-hexane, then add the 20-mL n-hexane rinse to the separatory funnel.

Hexane Extractable Gravimetric Method (continued)



5. Stopper the funnel. Invert the funnel and release the gases through the stopcock. Then, vigorously shake the funnel for two minutes.

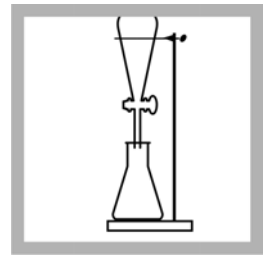
To release gases from the separatory funnel, invert it and shake it once very hard (support the stopper with your hand). Under a hood, point the delivery tube in a safe direction and **slowly** open the stopcock to release any gas. Close the stopcock. Repeat the venting procedure until you no longer hear the release of gases.



6. Let the funnel stand undisturbed for at least 10 minutes to ensure separation of the lower water layer and the upper solvent layer.

The solvent layer may be brown if a colored oil is present.

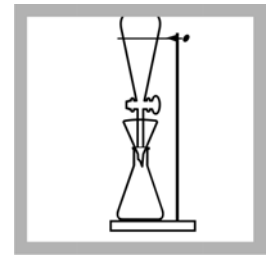
If you repeat this step the third time and the water layer is cloudy, allow the separatory funnel to stand undisturbed for 20 minutes for better separation of the water and solvent layers.



7. **Slowly** drain the lower water layer from the separatory flask into the original sample container or 500-mL volumetric flask. This should take about 3–4 minutes. Save the water layer for step 10.

To ensure that water is not transferred in step 9, allow several drops of solvent layer to drain into the water layer until the solvent layer is visible on top of the water.

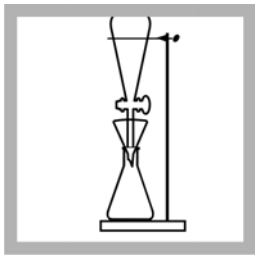
If the water layer drains too quickly, excess water will be present in the solvent layer. This causes sodium sulfate and water interference.



8. Set up the filtering funnel. Put the glass funnel in the neck of the distillation flask. Place a folded 12.5 cm filter paper in the funnel. Add 10 g of anhydrous sodium sulfate to the filter paper. Rinse the sodium sulfate with a small amount of the hexane. Discard the hexane properly.

Use the same filter, funnel and sodium sulfate when repeating this step for the second and third extractions. Remove large, hard sodium sulfate chunks between extractions to reduce sodium sulfate contamination.

Hexane Extractable Gravimetric Method (continued)



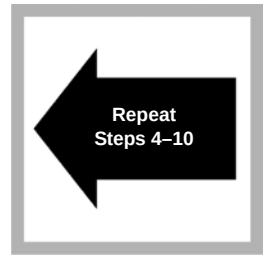
9. Drip-drain the solvent layer into the pre-weighed boiling flask through a funnel containing filter paper and 10 g anhydrous sodium sulfate. Gently stir the sodium sulfate with a glass stirring rod while the solvent layer is draining. Be careful not to rip the filter paper.

Any spillage will cause inaccurate results. To reduce spillage, use a glass rod to guide the sample solution into the filter.

10. Return the water layer to the separatory funnel.

Use the same glass funnel for the second and third extraction (referred to in step 11).

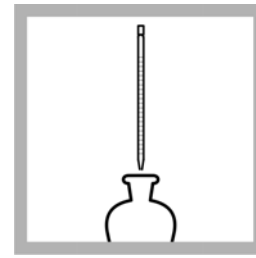
To reduce spillage, a second funnel can be used when pouring the water layer into the separatory funnel.



11. Repeat step 4 through step 10 two more times. After the third extraction, discard the water layer.

There may be small amounts of acetone and/or n-hexane in the water layer.

Refer to your local waste disposal procedure for proper disposal instructions.



12. Rinse the separatory funnel with three separate 5-mL aliquots of fresh n-hexane to remove any oil film left on the funnel walls. Drain each aliquot through the funnel containing the sodium sulfate into the distillation flask.

Hexane Extractable Gravimetric Method (continued)

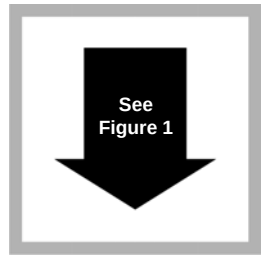
13. Rinse the tip of the glass funnel with 5 mL of n-hexane while removing it from the distillation flask. Check for sodium sulfate contamination.

Sodium sulfate contamination will appear as cubic crystals at the bottom of the distillation flask. If present, re-filter the solvent layer through filter paper without sodium sulfate. You must re-clean, dry and weigh the boiling flask and boiling chips; or have an extra flask ready in case this is necessary.

14. If HEM is to be determined, go to step 15.

If **only** the SGT-HEM is to be determined and the HEM is known, go to step 21.

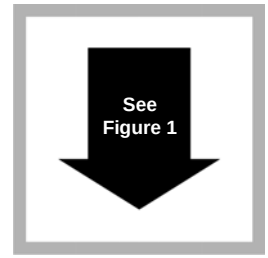
If only the SGT-HEM is to be analyzed, the HEM is needed in order to determine the amount of silica gel needed for the SGT-HEM. For each group of samples from a discharge, determine the HEM before the SGT-HEM.



15. Using the distillation assembly shown in Figure 13, distill off the n-hexane. Distillation is complete when there are no boiling bubbles or the distillation flask appears dry.

Use a steam bath or a hot plate to maintain a water bath at the proper temperature for the distillation. Do not place the flask directly on a hot plate. This will cause low results and is dangerous because n-hexane is volatile.

Evaporation will be faster if the long vertical arm of the connector is wrapped with insulation (paper towel, cloth or asbestos insulating tape). The distillation should take less than 30 minutes.



16. Disconnect the condenser/connector portion of the distillation assembly at the pinch clamp and remove the distillation flask from the heat source with an anti-lint cloth or tongs.

The distilled n-hexane may be re-used in future HEM extractions, but is not recommended for SGT-HEM due to the potential increased water content of the solvent.

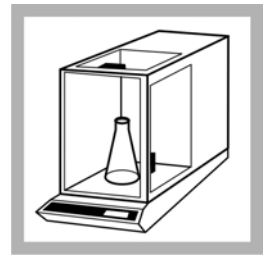
Hexane Extractable Gravimetric Method (continued)

17. Remove the remaining solvent vapors from the distillation flask by attaching the vacuum connector/gas inlet adapter to the flask. Apply a vacuum for 1–2 minutes or until all n-hexane solvent vapors have been removed.

Crystals on the bottom of the flask indicate that sodium sulfate may have been dissolved in the extraction steps. Re-dissolve the extract in n-hexane, filter into another pre-weighed flask and repeat steps 14–16. This is not necessarily true for the “standard” extraction since stearic acid is crystalline below 69 °C (156 °F). If sodium sulfate is present in the standard, big cubical crystals (not the flattened stearic acid crystals) will be visible. Also, an unusually high yield compared to the expected value will result.

18. Place the flask in a desiccator for 30 minutes (or longer if necessary) until it cools to room temperature.

If the silica gel indicator has turned red, replace the silica gel.



19. Using an analytical balance, weigh the flask to the nearest 0.1 mg. Record this weight. Do not touch the flask after weighing; fingerprints will add weight.

Always use a tong or a lint-free wipe when handling the flask.

Precise weighing is necessary for accurate results; multiple weight measurements are recommended. Re-wipe the flask before each measurement to ensure all contaminants are removed. Record each weight; use the lowest repeatable value for calculations.

20. Calculate the test results:

$A - B \times 1000 =$ mg/L HEM

A= Weight (mg) of residue

B= Weight (mg) of flask with boiling chips (step 3).

Example:

A = 92.4659 g

B = 92.4206 g

Sample volume = 0.350 L (350 mL)

$$\frac{92.4659 - 92.4206}{0.350} =$$

129.4 mg/L

If yield is less than 15 mg/L and additional precision is needed, use a 1-liter sample.

Hexane Extractable Gravimetric Method (continued)

21. If only calculating the HEM, stop here.

If continuing with SGT-HEM, re-dissolve residue with approximately 85 mL of fresh n-hexane. Heat slightly to ensure re-dissolving of all HEM materials.

22. For a 350-mL water sample, dilution is necessary if the HEM is above 2850 mg/L (for a 1-liter sample, dilute if the HEM is greater than 1000 mg/L).

To dilute to a 1000 mg/L sample, pour the re-dissolved HEM into a 100-mL volumetric flask. Rinse the distillation flask 3–4 times with 2–3 mL of n-hexane. Fill the volumetric flask to volume with n-hexane. Mix well. Into a 100-mL beaker, volumetrically pipet the amount (Va) determined by this equation:

$$V_a = \frac{10000}{W_h}$$

where:

V_a = Volume of aliquot to be withdrawn (mL) to get 1000 mg of HEM.

W_h = Weight of HEM (mg) (A –B x 1000 in step 19 (mg)). Dilute to about 100 mL with n-hexane.

23. Put a magnetic stir bar and the correct amount of silica gel (based on the equation below) into the flask with the solvent/ product from step 22.

$$\frac{3 \times \text{mg/L HEM}}{100} =$$

silica gel (g ± 0.3)

24. Stir solution on a magnetic stirrer for five minutes (minimum).

Hexane Extractable Gravimetric Method (continued)

25. Place a funnel on a clean, dry distillation flask with 3–5 boiling chips in it. Place a 12.5-cm filter paper in the funnel. Pre-moisten the filter paper with fresh n-hexane. Filter the solution through filter paper.

Rinse the beaker containing remaining silica gel 3 times with 5-mL aliquots of fresh n-hexane and pour the aliquots into the distillation flask.

26. Follow step 15–step 20. Weigh the product remaining in the bottom of the flask and calculate the results using the equation below:

$$\frac{A - B}{\text{Sample Volume}} = \text{mg/L SGT} - \text{HEM}$$

where:

A = Weight (mg) of residue

B = Weight (mg) of flask with boiling chips.

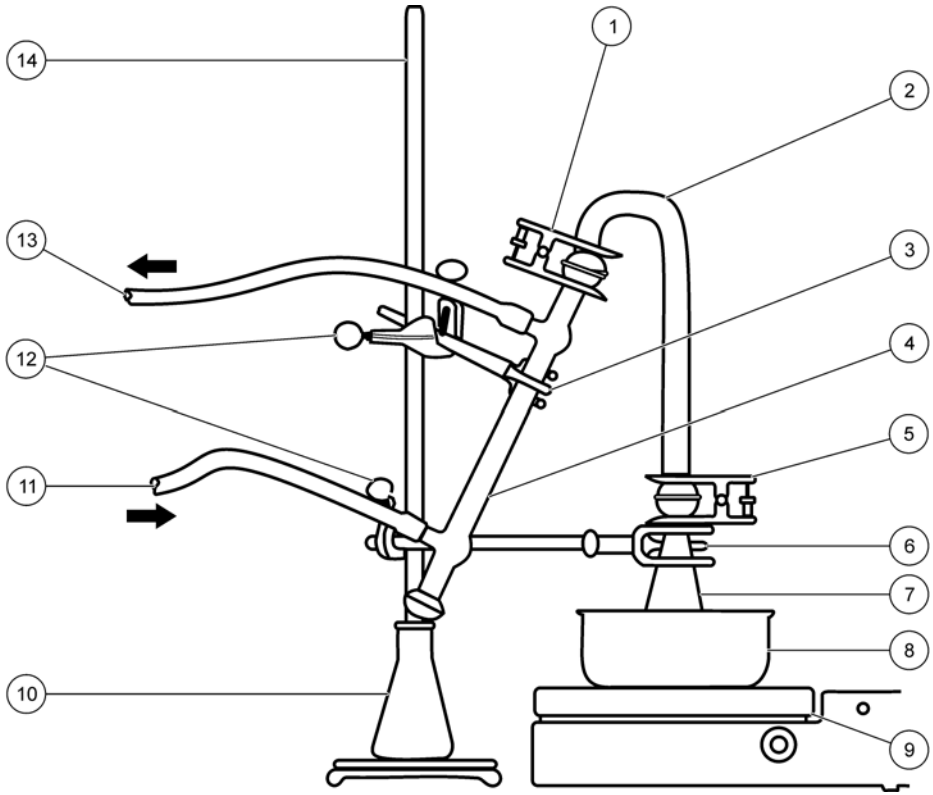


Figure 13 Distillation assembly

1. Pinch Clamp	**8.** Water Bath
2. J-Shaped Connector	**9.** Hot Plate
3. Clamp, 3-Prong	**10.** Receiving Flask
4. Condenser	**11.** Water In
5. Pinch Clamp	**12.** Clamp Holders
6. Clamp, 3-Prong	**13.** Water Out
7. Distillation Flask	**14.** Support Stand and Rod Assembly

Interferences

Substances extracted from samples will vary from source to source, depending upon the diversity of the site being sampled. Some samples may contain high amounts of detergents or particulates that can interfere with the extraction procedure. For these samples, it may be necessary to use a 350-mL sample rather than 1-liter (which is an option). In this circumstance, the 350-mL sample is EPA accepted for reporting. Wash all glassware in hot water with detergent, rinse with tap and distilled water and rinse with n-hexane or acetone.

If an emulsion forms between the two phases (at step 6) and is greater than one-third the volume of the solvent layer, filter the emulsion and solvent layer through a funnel with glass wool in it. If an emulsion still exists, other possible solutions include: stirring the solvent and emulsion layer with a stir bar, using solvent phase separation paper, centrifugation, using an ultrasonic bath with ice, adding NaCl or other physical methods. (Solid phase or other extraction techniques would fall under performance based modifications.)

A milky solvent/product layer in the distillation flask indicates water in the solvent layer. Let the flask stand one hour to allow the water to settle. Re-filter the solvent layer through sodium sulfate to remove remaining water.

Extremely low yields could mean a poor extraction (step 5 through step 8) and a high yield could indicate a problem in the solvent drying (step 8). Follow step 5 through step 8 very carefully and run your blank before you run samples in order to identify any possible interference due to these steps. If your blank indicates a yield above 1 mg per test, you should identify the source of contamination before continuing; likely sources are sodium sulfate contamination and improperly rinsed glassware.

Sample collection, preservation and storage

- Collect samples in wide-mouth glass bottles or directly in the separatory funnel for immediate analysis.
- Collection of sample may be done directly into the separatory funnel. Measure 350 mL of water with a graduated cylinder.
- Pour this into the separatory funnel. Use a laboratory pen to mark the 350-mL level. Fill with sample to this mark.
- Do not pre-rinse the bottle or separatory funnel with the sample.
- If analysis is delayed for more than a few hours, add 6 mL of 1:1 Hydrochloric Acid Solution for each liter or quart of sample.
- Check the pH to ensure it is 2 or less. Do not place the pH paper directly into the sample, but dip a glass rod into the sample and touch the pH paper with a drop of sample.
- Rinse the rod with n-hexane directly back into sample container after the pH has been determined to make sure that no product has adhered to the glass rod.
- If necessary, add more acid to bring pH below 2.
- Store the sample between 0–4 °C (32–39 °F). Preserved samples can be stored for 28 days.

Handling glassware

Before analysis, careful cleaning and drying of the glassware and boiling chips is necessary. Clean the chips and distillation flask by washing with hot water and detergent, rinsing with distilled water and then rinsing with acetone or n-hexane. Place the cleaned flask and boiling chips in a drying oven at 105–115 °C (220–240 °F) for 2 hours. Cool to room temperature in a desiccator for at least 30 minutes. Store in the desiccator until needed.

To eliminate errors, always handle the flask with tongs or an anti-lint wipe. If the same flasks are used repeatedly, record their weights after drying in the oven without boiling chips. The drying step

may be skipped if the flasks weigh the same after the acetone or n-hexane rinse as it does after drying. Boiling chips will vary in weight; their weight should be added to the flask weight.

Definition of HEM and SGT-HEM

Oil and grease is the conventional term used to define pollutants of this nature. The new term *n-Hexane Extractable Materials* (HEM) indicates this method may be applied to materials other than oils and greases.

Likewise, the term *Total Petroleum Hydrocarbons* (TPH) was traditionally used to classify aliphatic hydrocarbon materials. The new term *Silica Gel Treated n-Hexane Extractable Material* (SGT-HEM), indicates that this method may be applied to materials other than aliphatic petroleum hydrocarbons that are not adsorbed by silica gel.

Detection limit

This method is not applicable to measurements of materials that volatilize at temperatures below approximately 85 °C (185 °F). Petroleum fuels from gasoline through #2 fuel oil may be partially lost in the solvent removal operation. Some crude oils and heavy fuel oils contain a significant percentage of materials that are not soluble in n-hexane. Recoveries of these materials may be low.

The method is capable of measuring HEM and SGT-HEM in the range of 15 to 3000 mg/L when using a 350-mL sample and may be decreased to as low as 5 mg/L if a 1-liter sample is used. When using the 1-liter sample volume, use the amount of reagents listed for the 1-liter size in *EPA monitoring, testing procedures and modifications*.

Standard preparation

1. Transfer 400 ±4 mg stearic acid and 400 ±4 mg hexadecane into a 100-mL volumetric flask.
2. Pour 75 mL of acetone into the flask. Cover with a small beaker and stir gently. Heat slightly until all material is in solution. Over-heating with the lid on causes pressure build up.
3. Fill to volume with acetone. Cover. Allow to equilibrate to room temperature and continue to fill to volume until solution is at stable volume.
4. Using a volumetric pipet, transfer 5 mL of the solution from step 3 into 350 mL of deionized reagent water. This standard solution should be 114.3-mg/L HEM or 57.1-mg/L SGT-HEM. If using a 1-liter water sample, 5 mL gives concentrations of 40 mg/L HEM and 20 mg/L SGT-HEM. To verify the concentration, pipette 5 mL of the solution from step 3 in a pre-weighed flask, place in hood and allow acetone to evaporate off. Weigh the flask. Verify that the weight difference before and after solution addition is 40 (±1) mg.

EPA monitoring, testing procedures and modifications

If the Oil and Grease tests are used for compliance reporting to the USEPA, make the following changes to the procedure:

1. Use a 1-liter sample in a 2000-mL separatory funnel rather than a 350-mL sample in a 500-mL separatory funnel (step 1).
2. Use 6 mL (instead of 4 mL) of 1:1 hydrochloric acid to bring the pH below 2 (step 2) and 30 mL of n-hexane instead of 20 mL of n-hexane for the extraction (step 4).

Before testing real samples for oil and grease, you must be able to obtain a MDL (Minimum Detection Limit) less than or equal to the EPA reported MDL and to report an IPR (Initial Precision and Recovery). **Before attempting the MDL and IPR, it is highly recommended to run laboratory reagent water blanks to eliminate all interference.**

MDL: The USEPA-documented MDL is extremely difficult to reproduce if the slightest sodium sulfate contamination occurs. EPA Method 1664 requires the MDL to be ≤ 1.4 mg/L for HEM and ≤ 1.6 mg/L for SGT-HEM. This is calculated by determining the standard deviation of seven standard samples for HEM or seven standards for SGT-HEM and multiplying their respective standard deviations by 3.143 (Student's t test).

The recommended standard concentration for determining the MDL is about 5 mg/L. To prepare the standard for HEM follow steps 1–3 in *Standard preparation*, but change step 1 to transfer 100 (± 4) mg stearic acid and 100 (± 4) mg hexadecane to a 250-mL volumetric flask. To prepare the SGT-HEM standard, transfer 200 (± 4) mg of decahexane only into a 250-mL volumetric flask. Transfer 5 mL of either standard into 1-liter of reagent water. Analysis of the standard should give 5 mg/L for either HEM or SGT-HEM.

IPR: Follow the procedure for HEM and SGT-HEM, using deionized water (void of any oil and grease) as the blank. Perform the procedure four separate times using 5 mL of the standard (40 mg/L 1:1 stearic acid/hexadecane) diluted into 1 liter of demineralized water. Report the average percent recovery (X) and the standard deviation of the percent recovery(s) for both HEM and SGT-HEM. X and s should fall within these parameters:

- HEM: Precision(s) ≤ 10%; Recovery(X) 83–101%
- SGT-HEM: Precision(s) ≤ 13%; Recovery(X) 83–116%

If not within these ranges, correct the problem and repeat IPR.

Once the MDL and IPR have been established, keep records for EPA verification or for future reference.

Oil and grease reporting to EPA

To report the HEM and/or SGT-HEM, include the following data for each set of up to ten samples.

1. **BLANK:** Must be less than 5.0 mg/L for HEM and SGT-HEM.

   Note: *For compliance monitoring, it may be necessary to use a standard that either matches the regulatory concentration limit or is 1 to 5 times higher than the concentration of the sample (B), whichever is greater. The concentration of the spike (T) needs to be divided by 2 for SGT-HEM if using the standard (40 mg/L 1:1 stearic acid/hexadecane).*

2. **OPR (Ongoing Precision and Recovery):** Determine the amount of analyte in a one liter sample containing 5 mL of the standard (40 mg/L 1:1 stearic acid/hexadecane). Continue on if the HEM recovery is 70–114% and the SGT-HEM recovery is 66–114%. If recovery is lower, an interference may be present or analysis technique may be faulty. Identify the cause and repeat OPR until within range.

3. **MS and MSD (matrix spike and matrix spike duplicate):** Determine the background concentration (B) of your sample by running HEM and SGT-HEM for the discharge water. Spike two 1-liter discharge water samples with 5 mL of standard and measure the concentration of the analyte after spiking (A).

   Determine the Percent Recovery (P) as follows:

   $$P_{HEM(40\ mg/L)} = \frac{100 \times (A - B)}{T}$$

   $$P_{SGT-HEM} = \frac{100 \times (A - B)}{T/2}$$

   If HEM Recovery is 79–114% and SGT-HEM Recovery is 66–114%, then calculate the Relative Percent Difference (RPD). If recovery is lower, an interference may be present. Identify and correct the interference, then repeat MS and MSD.

   $$RPD = \frac{Conc_{MS} - Conc_{MSD}}{Conc_{MS} + Conc_{MSD}} \times 200$$

If RPD for HEM ≤ 18 and RPD for SGT-HEM ≤ 24, then continue with step 4.

If recovery is lower, an interference may be present. Identify and correct the interference, then repeat MS and MSD.

After every five MS/MSD tests, compute the average percent recovery (Pa) and standard deviation of the percent recovery (sp). Record these numbers as Pa ±2sp.

4. Calibrate your analytical balance at 2 mg and 1000 mg using class “S” weights to ensure calibration within ±10%.

5. Analyze up to 10 samples from the source used in the MS and MSD before starting back at step 1. For every 10 samples you must determine a new blank, OPR, RPD, MS and MSD.

Summary
Each laboratory must first verify the MDL and IPR and ensure they are within proper parameters before reporting oil and grease test results to the EPA. Once this is established for a laboratory, it does not need to be done again.

For every 10 samples per discharge source, one must calibrate the balance, report one blank, one OPR, one MS and one MSD. Logs must be kept on percent recovery and relative percent differences for MS/MSD tests. For every five MS/MSD test, calculate and record the average percent recovery and standard deviation.

Summary of method

Oil and Grease & Total Petroleum Hydrocarbons (TPH) include any material that may be recovered as a substance that is soluble in the n-hexane extractant. These include substances such as relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related materials. When measuring oil and grease (HEM) gravimetrically, the substances are extracted from the sample with n-hexane, then the n-hexane is evaporated. The residue left is weighed to determine the concentration of oil and grease materials in mg/L.

When measuring Total Petroleum Hydrocarbons (SGT-HEM) gravimetrically, the substances are extracted from the sample with n-hexane, then mixed with silica gel to absorb non-TPH components. Then the n-hexane is evaporated. Like the HEM, the residue left is weighed to determine the concentration of total petroleum hydrocarbons.

Note: *Careful technique is required to obtain accurate and precise results.*

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hydrochloric Acid Standard Solution, 1:1 (6N)	4 mL	500 mL	88449
Hexane, ACS grade	100–200 mL	500 mL	1447849
pH Paper, 0–14 pH units	varies	100/pkg	2601300
Silica Gel with indicator (for desiccator)	varies	454 g	1426901
Silica Gel, 100–200 mesh	1–30 g	500 g	2665034
Sodium sulfate, anhydrous	10 g	113 g	709914

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Adapter, vacuum connector/gas inlet, 28/15	1	each	1433900
Aspirator, vacuum	1	each	213100
Balance, Analytical, 115 VAC 60 Hz.	1	each	2936801
Boiling Chips	3–10	500 g	2055734
Clamp, 3-prong	2	each	42200
Clamp, holder	2	each	32600
Clamp, pinch type, No. 28, F/Glass Joints	2	each	1433800
Condenser, reflux, w/ground glass joints, 28/15	1	each	1433700
Cylinder, graduated, 500-mL	1	each	2088549
Cylinder, graduated, 50-mL	1	each	50841
Desiccator	1	each	2088800
Desiccator Plate	1	each	1428400
Filter Funnel, 65-mm, short stem	1	each	2664700
Filter Paper, 12.5-cm, folded, pore size 8 to 12 µm	1	100/pkg	69257
Flask, Erlenmeyer, 125-mL	1	each	50543
Flask, Erlenmeyer, 125-mL, w/ground glass joint 28/15	2	each	1434000
Funnel, separatory, 500-mL	1	each	52049
Marker, Laboratory	1	each	2092000
Oven, drying, 120 VAC, 50 Hz	1	each	1428900
Pipette filler, safety bulb	1	each	1465100
Pipette, serological, 5-mL	1	each	53237
Ring Support, 4-inch	1	each	580-01
Rod, glass	1	3/pkg	177001
Steam bath, 8-inch, 5-ring	1	each	2347900
Hot Plate/Stirrer, 7.25 x 7.25", 120 VAC	1	each	2881600
Stir Bar, 22.2 x 7.9 mm	1	each	2095350
Support stand	2	each	56300
Tongs, crucible, 9-inch	1	each	56900
Tube, connecting, J-shaped, w/ground glass joint, 28/15	1	each	1814300
Tubing, black rubber, 7.9 X 2.4 mm	1	3.6 m	56019

Recommended standards

Description	Unit	Catalog number
Hexadecane, 99%, 400 mg	100 mL	2664842
Stearic Acid, 400 mg	500 g	2664934

Optional reagents and apparatus

Description	Unit	Catalog number
Acetone, ACS	500 mL	1442949
Separatory funnel, 2-liters	each	52054
Beaker, 50 mL	each	50041H
Balance, Weight Set	each	2617601
Hotplate/Stirrer 7.25 x 7.25 in., 220–240 VAC	each	2881602
Ring Support, 4.5 in.	each	2656300
Furnace, Muffle, 120 VAC	each	1429600
Pipet, Volumetric 5.0 mL	each	14515-37
Flask, Volumetric	100 mL	14574-452
Cylinder, graduated, 100 mL	each	508-42

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Edition 7

Oil and Grease

DOC316.53.01186

USEPA[1] Solid Phase Extraction Method

Method 10300

5 to 1000 mg/L HEM and SGT-HEM

Hexane Extractable Gravimetry

Scope and Application: For water and wastewater

[1] Equivalent to USEPA Method 1664A, Solid Phase Extraction (SPE)

Test preparation

Before starting the test:

Follow the instructions for sample collection and acidification in *Sample collection, preservation and storage*.
Assemble the Xenosep® SPE apparatus as shown in Figure 14. Be sure to put the waste collection tube in the large flask before installing the funnel assembly. Put the pattern side of the SPE filter down in the SPE filter support (Figure 15).
Wash all glassware in hot water with detergent, rinse with tap and distilled water and rinse with acetone or n-hexane.
Rinse all glassware thoroughly with hexane to make sure the analyte is not retained on the apparatus. Inadequate rinsing will result in low recovery.
Determine a blank value (1 liter of distilled or deionized water) with each new lot of reagents. If the blank result is greater than 5 mg, resolve the source of error or remove interferences before performing this procedure.
If analyzing for SGT-HEM, rinse the inner cavity of the aluminum dish with a few milliliters of acetone and then with a few milliliters of hexane to remove any potential artifacts.
Label the aluminum dish and heat in an oven for one hour at 105 °C. Cool the dish in a dessicator for 30 minutes. Weigh the dish on an analytical balance to the nearest 0.1 mg. Record this weight as "B" for HEM step 22 or SGT-HEM step 12.
Make sure that the aluminum dish is removed from the hot plate before the solution has completely evaporated. Heating the solution too long will result in low recovery.
Use a vacuum pump that can generate a Free Air Flow of at least 1 CFM (3 CFM preferred). Insufficient SPE disk drying will result in low recovery.

Collect the following items :

Description	Quantity
Hydrochloric acid, 1:1	6 mL
Hexane (n-hexane) in Teflon-FEP wash bottle	1 wash bottle
Methanol in PE wash bottle	1 wash bottle
Deionized water in PE wash bottle	1 wash bottle
SPE Starter Kit, EPA Method 1664A	1 kit
SPE Consumables Kit	1 kit
SPE Solvent Recovery Kit	1 kit
Aluminum weighing dish	1
Hot plate	1
Vacuum pump	1

Collect the following items (continued):

Description	Quantity
pH paper, 0–14 pH units	1
Lab stand	1
Clamp, swivel	2
Desiccator	1
For SGT-HEM:	
Silica gel	1 bottle
125-mL Erlenmeyer flask	1
100-mL volumetric flask (for HEM results over 1000 mg/L)	1
Magnetic stir plate	1
Aluminum weighing dish	1
Additional sodium sulfate column	1

See *Consumables and replacement items* for reorder information.

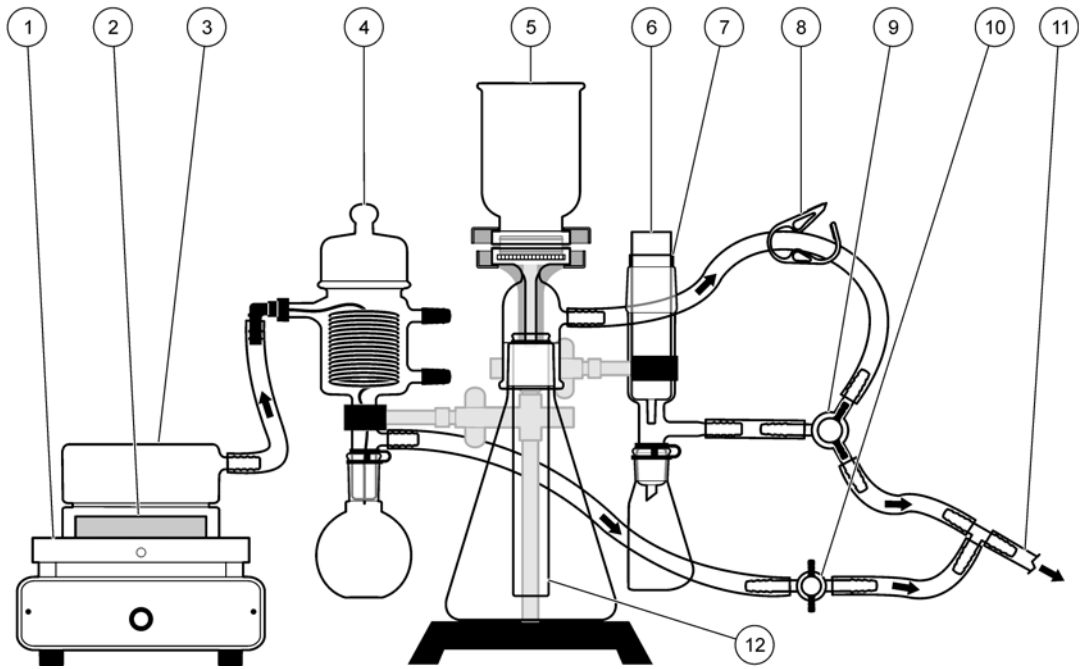


Figure 14 Solid phase extraction assembly

1. Hot plate	**7.** Eluter tube
2. Aluminum dish	**8.** Clamp, tubing
3. Glass dome	**9.** 3-way valve

4. Solvent recovery assembly	**10.** 2-way valve
5. Funnel assembly (see Figure 15)	**11.** To vacuum
6. Sodium sulfate column	**12.** Waste collection tube with O-Ring

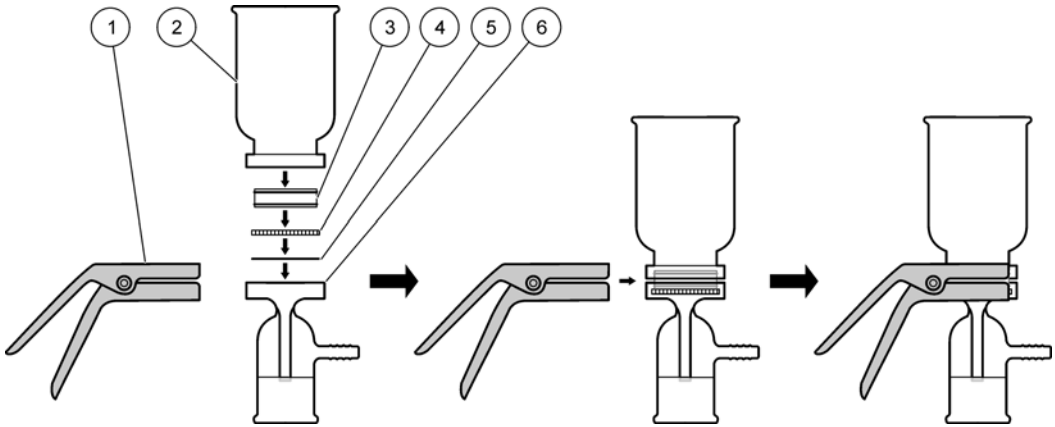


Figure 15 Funnel assembly

1. Aluminum clamp	**4.** SPE filter—pattern side down
2. Funnel	**5.** Stainless steel support
3. Coupler with O-Rings	**6.** SPE starter holder

Solid phase extraction for HEM

1. Add approximately 10 mL of hexane to the funnel. Wait 5 seconds. Turn the vacuum on and off to pull the solvent into the waste collection tube.

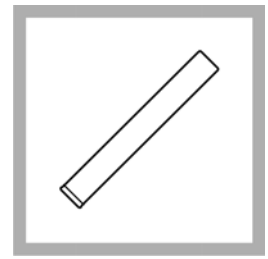
Repeat with a second 10-mL portion of hexane.

Make sure the valves are set to pull vacuum from the funnel holder and the tubing clamp is open.

2. Turn the vacuum on for 1 minute to dry the filter. Turn off the vacuum.

3. Add approximately 10 mL of methanol to the funnel. Wait 5 seconds. Turn the vacuum on and off to pull the solvent into the waste collection tube.

Do not allow the filter to become dry.



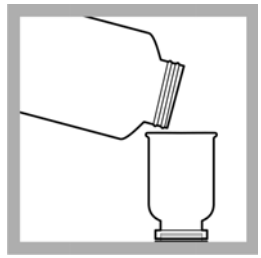
4. Remove the tube and discard the solvent waste. Refer to the MSDS and local regulations for safe disposal of the solvent.

Solid phase extraction for HEM (continued)

5. Add approximately 20 mL of deionized water to the funnel. Wait 5 seconds. Turn the vacuum on and off to pull the water into the flask.

Do not allow the filter to become dry.

If the filter becomes dry, repeat steps 3–5.

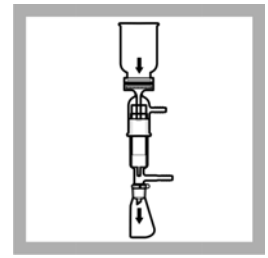


6. Slowly pour the acidified sample into the funnel and turn on the vacuum. Use deionized water to rinse any debris from the walls of the funnel.

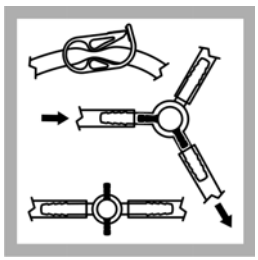
See *Sample collection, preservation and storage* for sample acidification.

7. Leave the vacuum on for 4 to 8 minutes to air dry the filter. Turn off the vacuum.

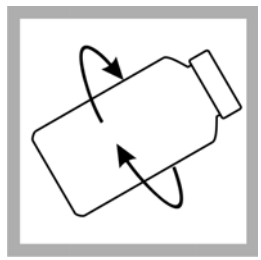
Do not leave the vacuum on for more than 8 minutes.



8. Put the funnel assembly on the eluter tube.



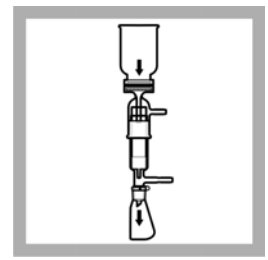
9. Turn the valve to apply vacuum to the eluter tube. Close the tubing clamp on the funnel holder tube.



10. Add 10 mL of hexane to the empty sample bottle. Shake the bottle in a horizontal, circular motion for 10 seconds to rinse the bottle.

11. Use a transfer pipet to collect the hexane from the shoulder of the sample bottle. Slowly rinse the walls of the funnel with the hexane. Go around the funnel at least 3 times.

Repeat steps 10–11 two more times.

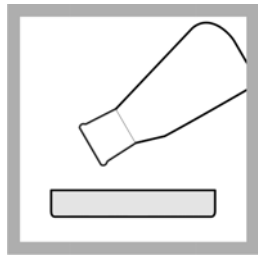


12. Turn the vacuum on and off to pull the solvent into the flat-sided flask.

Rinse the walls of the funnel with approximately 10 mL of hexane. Wait 5 seconds. Turn the vacuum on and off.

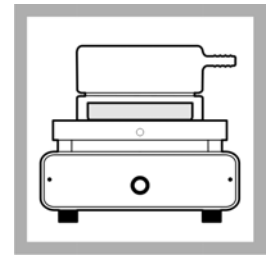
Solid phase extraction for HEM (continued)

13. Remove the flat-sided flask from the eluter tube.

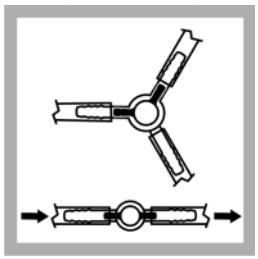


14. Pour the hexane into a pre-weighed aluminum dish.

15. Rinse the flat-sided flask with approximately 5 mL of hexane. Add the hexane to the dish.

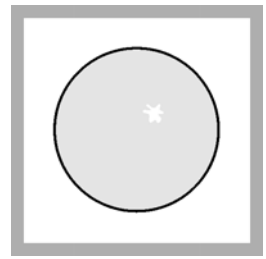


16. Put the dish on the hot plate and place the glass cover over the dish.



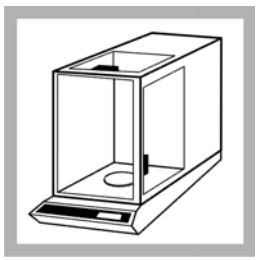
17. Close the 3-way valve and open the 2-way valve to pull vacuum from the solvent recovery apparatus.

18. Turn on the vacuum. Turn the hot plate on low heat (35–85 ºC). Heat for approximately 2 minutes.



19. Examine the dish for dry spots. As soon as there is a dry spot remove the dish from the hot plate. Put the dish in a fume hood until the rest of the hexane has evaporated.

20. When the dish is dry, put the dish in a desiccator for 30 minutes.



21. After 30 minutes, weigh the dish to the nearest 0.1 mg. Repeat steps 20 and 21 until the weight loss is less than 0.5 mg from the previous weight. Record the weight.

22. Calculate the test results:

$$\frac{A - B}{\text{sample volume}} \times 1000$$

A= weight of dish with residue (g) (step 21)

B= weight of dish (g)

Example:

A= 6.2394 g

B= 6.2318 g

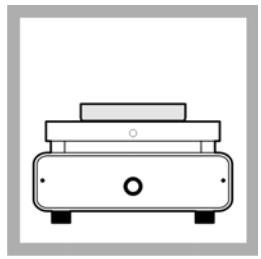
sample volume = 0.950 L

$$\frac{6.2394\ g - 6.2318\ g}{0.950\ L} \times 1000 =$$

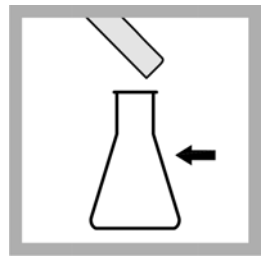
8.0 mg/L HEM

Solid phase extraction for SGT-HEM (HEM < 1000 mg/L)

1. Add hexane to the residue in the aluminum dish from the HEM test until the dish is approximately half full (approximately 60 mL).



2. Heat slightly to dissolve all of the residue.



3. Pour the mixture into a 125-mL Erlenmeyer flask. Rinse the pan and funnel several times with hexane and add to the flask. Add hexane to approximately the 100-mL mark.

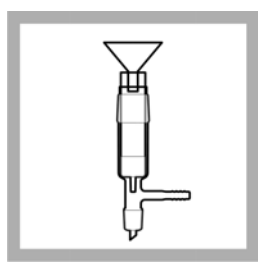
4. Add 3 (± 0.3) g of silica gel for every 100 mg of HEM or fraction thereof:

$$\frac{3 \times \text{mg/L HEM}}{100} = \text{silica gel (g)}$$

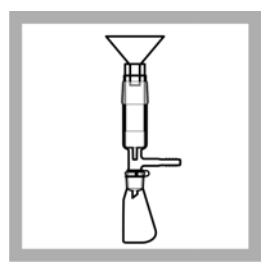
Example: if the HEM value was 735 mg, round 7.35 g up to the next whole gram increment (8 g or 800 mg) and add 3 x 800/100= 24 g of silica gel.

Do not add more than 30 g silica gel.

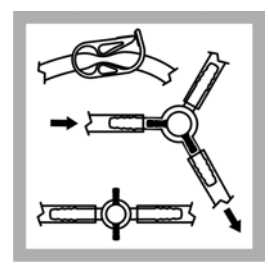
5. Add a stir bar to the flask and stir for 5 minutes.



6. Put a new sodium sulfate column into the eluter tube. Put the funnel on the eluter tube.



7. Install a clean flat-sided flask.



8. Adjust the valves to pull vacuum from the eluter tube.

Solid phase extraction for SGT-HEM (HEM < 1000 mg/L) (continued)

9. Pour the solution from the Erlenmeyer flask into the funnel. Turn the vacuum on and off to pull the solution into the flat-sided flask.

10. Rinse the Erlenmeyer flask with 5 mL hexane. Add the hexane to the funnel. Turn the vacuum on and off.

Rinse the walls of the funnel with approximately 5 mL of hexane. Turn the vacuum on and off.

11. Complete steps 13–21 under *Solid phase extraction for HEM* to evaporate the solvent.

12. Calculate the test results:

$$\frac{A - B}{\text{sample volume}} \times 1000$$

A= weight of dish with residue (g) (step 21)

B= weight of dish (g)

Example:

A= 6.2360 g

B= 6.2320 g

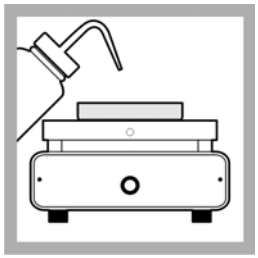
sample volume = 0.950 L

$$\frac{6.2360\ g - 6.2320\ g}{0.950\ L} \times 1000 =$$

4.2 mg/L SGT-HEM

Report result as ≤5 mg/L SGT-HEM

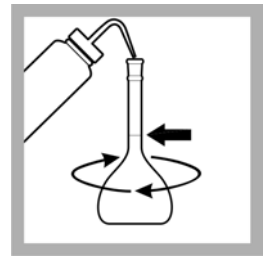
Solid phase extraction for SGT-HEM (HEM > 1000 mg/L)



1. Add hexane to the residue in the aluminum dish from the HEM test until the dish is approximately half full (approximately 60 mL).

Heat slightly to dissolve all of the residue.

2. Pour the mixture into a 100-mL volumetric flask. Rinse the pan and funnel several times with hexane and add to the flask.



3. Dilute to the mark with hexane and mix well.

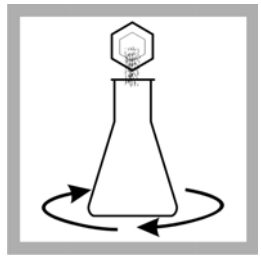
4. Calculate the volume to remove for the silica gel treatment:

$$V_{1000} = \frac{100{,}000}{\text{HEM value}}$$

V_{1000} = volume that contains 1000 mg HEM

Solid phase extraction for SGT-HEM (HEM > 1000 mg/L) (continued)

5. Remove the amount calculated in step 4 from the volumetric flask and add it to a 125-mL Erlenmeyer flask. Add hexane to approximately the 100-mL mark.



6. Add 30 g of silica gel to the flask.

If the volume added in step 5 contained less than 1000 mg HEM, calculate the amount of silica gel to add based on the mg HEM that was added to the flask.

$$\frac{3 \times \text{mg HEM in aliquot}}{100} = \text{silica gel (g)}$$

7. Complete steps 5–12 in the procedure for *Solid phase extraction for SGT-HEM (HEM < 1000 mg/L)*.

8. Correct the test result for the reduced volume that was treated with the silica gel:

$$W_d \times \frac{100}{V_{1000}} = W_c$$

where:

W_d = result from step 12

V_{1000} = volume removed for silica gel treatment

W_c = corrected SGT-HEM result

Interferences

For samples that are known to contain extremely high levels of oil and grease, use a smaller sample volume and correct for the volume difference to give the result as a 1-L sample.

High concentrations of particulates in the water sample can clog the SPE filter or retain high levels of water, which can lower the extraction efficiency. Inorganic particulates are easier to filter than organic particulates. The following techniques may help to filter samples containing high levels of particulates:

- Decanting—allow the sample to settle and pour the top portion into the funnel first. Just before dryness, add the rest of the sample. Remove any sediment from the bottle and add it to the SPE filter. Use deionized water to rinse any sediment that remains in the bottle.
- Prefilters or prefilter fibers—place the prefilter or prefilter fibers into the coupler before the funnel is attached.
- Drying agents—add magnesium sulfate to the SPE filter or to the prefilter to remove water that may be trapped in the particulates.
- Filtration aids—add materials such as sodium chloride, sand, diatomaceous earth or glass beads to help speed the complete filtration of samples that contain organic particulates.

This method is not applicable to materials that volatilize at temperatures below approximately 85 °C (185 °F). Petroleum fuels from gasoline through #2 fuel oil may be partially lost in the solvent removal operation. Some crude oils and heavy fuel oils contain a significant percentage of materials that are not soluble in n-hexane. Recoveries of these materials may be low.

Sample collection, preservation and storage

Collect 1 L (950–1050 mL) of sample in a wide-mouth glass bottle. Do not rinse the bottle with sample before collection. The sample must be at room temperature before the test.

Sample volume
Complete the steps that follow to measure the sample volume.

6. Use a laboratory pen to mark on the sample bottle the liquid level of the sample.
7. When the test is complete, fill the bottle to this mark with tap water.
8. Pour the tap water into a 1-L graduated cylinder and write down the volume. Use this volume for the sample volume in the final step of the test procedure for HEM or SGT-HEM.

Acidification
The sample must be acidified with 1:1 hydrochloric acid (HCl) solution to a pH of 2 or less before the test is started. To find the volume of HCl to use, collect a separate aliquot, add HCl until the pH is less than 2 and add this volume of acid to each sample bottle before collection. Do not dip pH paper, a pH electrode, a glass rod or other materials into the sample because oil and grease in the sample may adhere to these items.

If the test can not be started for more than a few hours after collection, cool and store the sample at 0–4 °C (32–39 °F). Acidified samples can be stored for 28 days.

Accuracy check

For USEPA reporting, each laboratory must first measure an acceptable MDL (Minimum Detection Limit) and IPR (Initial Precision and Recovery) before samples can be measured. Before measuring the MDL or IPR, run laboratory reagent water blanks to eliminate interferences.

Standard solution preparation
Required items:

- Stearic acid, 98% minimum
- Hexadecane, 98% minimum
- Acetone for Organic Residue Analysis, residue less than 1 mg/L
- 100-mL Class A volumetric flask
- 10.0-mL Class A volumetric pipet
- 15.0-mL Class A volumetric pipet

1. Put 200 (± 2) mg stearic acid and 200 (± 2) mg hexadecane into a 100-mL volumetric flask.
2. Add 75–85 mL of acetone and shake vigorously until all material has dissolved.
3. Allow to equilibrate to room temperature and fill to volume with acetone. Mix well. The concentration of this stock solution is 4000 mg/L HEM (2000 mg/L SGT-HEM).
4. Use a volumetric pipet to dilute the stock solution for use in MDL, IPR or OPR measurements:

   MDL standard solution:

   a. Add 15 mL of the stock solution into a clean 100-mL volumetric flask. Dilute to the mark with acetone and mix well.
   b. Pipet 10 mL for HEM (or 20 mL for SGT-HEM) into a 1-L volumetric flask. Dilute to the mark with deionized water at pH < 2 and mix well. The concentration of this standard solution is 6 mg/L HEM (or 6 mg/L SGT-HEM).

IPR or OPR standard solution:

Add 10 mL of the stock solution into 1 liter of deionized water and mix well. The concentration of this solution is 40 mg/L HEM (20 mg/L SGT-HEM).

Note: *To verify the concentration of the IPR/OPR standard, use a pipet to add 10 mL of the standard solution into a pre-weighed flask or dish. Allow the acetone to evaporate in a fume hood. When dry, weigh the flask or dish. The weight of the residue should be 40 (±1) mg.*

EPA requirements for MDL and IPR

MDL: measure seven replicates of a 6 mg/L standard solution, find the standard deviation and multiply the standard deviation by 3.143 (Student's t test). The acceptable limits are:

- HEM: ≤ 1.4 mg/L
- SGT-HEM: ≤ 1.6 mg/L

IPR: follow the procedure for HEM and SGT-HEM (if necessary) four separate times using a 40 mg/L HEM (20 mg/L SGT-HEM) standard solution. Report the average percent recovery (x) and the standard deviation for both HEM and SGT-HEM. The acceptable limits are:

- HEM: Precision(s) ≤ 11%; Recovery (x) 83–101%
- SGT-HEM: Precision(s) ≤ 28%; Recovery (x) 83–116%

A low result can be an indication of loss during quantitative transfers or loss during heating. A high result can be an indication of incomplete drying or contamination. Run a blank before running samples. If the blank result is more than 5 mg, identify and remove the source of contamination.

After acceptable values are obtained for the MDL and IPR, keep records for USEPA verification.

Oil and grease reporting to USEPA

Include the following data with the HEM and/or SGT-HEM results for each set of up to 20 samples per discharge source.

1. **Blank value:** must be less than 5.0 mg/L for HEM and SGT-HEM.

   Note: *It may be necessary to use a standard that matches the regulatory concentration limit, is 1 to 5 times higher than the concentration of the sample (B) or is at the concentration of the Ongoing Precision and Recovery, whichever is highest. Divide the concentration of the spike (T) by 2 for SGT-HEM if using the 40 mg/L HEM (20 mg/L SGT-HEM) standard.*

2. **OPR (Ongoing Precision and Recovery):** add 10 mL of the 40 mg/L HEM (20 mg/L SGT-HEM) standard to a 1-liter sample and run the test. The acceptable limits for recovery are:

   - HEM: 78–114%
   - SGT-HEM: 64–132%

3. **MS and MSD (matrix spike and matrix spike duplicate):** measure the HEM and SGT-HEM concentration of the sample (B). Spike two 1-L samples with 10 mL of the 40 mg/L HEM (20 mg/L SGT-HEM) standard and measure the concentration after spiking (A).

   Calculate the Percent Recovery (P) as follows:

   $$P_{HEM\ (40\ mg/L)} = \frac{100 \times (A - B)}{T}$$

   $$P_{SGT-HEM} = \frac{100 \times (A - B)}{T/2}$$

   where:
   A = concentration of the unspiked sample
   B = concentration of the spiked sample
   T = concentration of the spike solution

If the recovery for HEM and SGT-HEM is within the acceptable limits for OPR (step 2), then calculate the Relative Percent Difference (RPD).

$$\text{RPD} = \frac{\text{Conc}_{\text{MS}} - \text{Conc}_{\text{MSD}}}{\text{Conc}_{\text{MS}} + \text{Conc}_{\text{MSD}}} \times 200$$

If the RPD for HEM is ≤ 18 and for SGT-HEM ≤ 34, then continue with step 4. If the recovery is lower than the RPD, an interference may be present. Identify and correct the interference, then repeat MS and MSD.

After every five MS/MSD tests, calculate the average percent recovery (Pa) and standard deviation of the percent recovery (sp). Record these numbers as Pa ±2sp. Update the accuracy assessment on a regular basis (e.g., after five to ten new accuracy measurments).

4. **Balance calibration:** measure a 2 mg and a 1000 mg class “S” weight on the analytical balance before and after each analytical batch. If the values are not within 10% of the actual weight, recalibrate the balance.

Summary of method

Oil and Grease & Total Petroleum Hydrocarbons (TPH) include any material that may be recovered as a substance that is soluble in the n-hexane extractant. These include substances such as relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related materials. When measuring oil and grease (HEM) gravimetrically, the substances are extracted from the sample with n-hexane, then the n-hexane is evaporated. The residue left is weighed to determine the concentration of oil and grease materials in mg/L.

When measuring Total Petroleum Hydrocarbons (SGT-HEM) gravimetrically, the substances are extracted from the sample with n-hexane, then mixed with silica gel to absorb non-TPH components. Then the n-hexane is evaporated. Like the HEM, the residue left is weighed to determine the concentration of total petroleum hydrocarbons.

Definition of HEM and SGT-HEM
Oil and grease is the conventional term used to define pollutants of this nature. The newer term *n-Hexane Extractable Materials* (HEM) indicates this method may be applied to materials other than oils and greases.

Likewise, the term *Total Petroleum Hydrocarbons* (TPH) was traditionally used to classify aliphatic hydrocarbon materials. The newer term *Silica Gel Treated n-Hexane Extractable Material* (SGT-HEM), indicates that this method may be applied to materials other than aliphatic petroleum hydrocarbons that are not adsorbed by silica gel.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Hydrochloric Acid Standard Solution, 1:1 (6 N)	6 mL	500 mL	88449
Hexane, for Organic Residue Analysis	100–200 mL	1 L	2510253
Methanol, ACS grade	10 mL	500 mL	1446449
Silica Gel, 100–200 mesh (for SGT-HEM)	1–30 g	500 g	2665034

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Balance, Analytical, 115 VAC 60 Hz.	1	each	2936801
Bottle, wash, 500-mL Teflon, FEP	1	each	2505100
Bottle, wash, 500-mL	1	3/pkg	2927204
Bottle, wide-mouth, 1-L	1	each	2951401
Clamp, swivel	2	each	2503400
Cylinder, graduated, 1-L	1	each	108153
Desiccator	1	each	2088800
Desiccator Plate	1	each	1428400
Flask, Erlenmeyer, 125-mL (for SGT-HEM)	1	each	50543
Hot Plate (Thermolyne), 120 VAC, 50 Hz	1	each	2344100
Marker, laboratory	1	each	2092000
Oven, drying, 120 VAC, 50 Hz	1	each	1428900
Pump, vacuum, 27 in. Hg, 1.3 CFM	1	each	2824800
SPE Consumables Kit	1	24/pkg	2943800
SPE Starter Kit, EPA Method 1664A	1	each	2943231
SPE Solvent Recovery Kit	1	each	2514300
Stirrer, magnetic, 120 VAC	1	each	2343600
Stir Bar, 22.2 x 7.9 mm	1	each	2095350
Support stand	1	each	2504900
Tongs, crucible, 9-inch	1	each	56900

Recommended standards

Description	Unit	Catalog number
Hexadecane, 99%	100 mL	2664842
Stearic acid	500 g	2664934

Optional reagents and apparatus

Description	Unit	Catalog number
Acetone, for Organic Residue Analysis	1 L	2510153
Flask, volumetric, Class A, 100-mL	each	1457442
Flask, volumetric, Class A, 1-L	each	1457453
pH Paper, 0–14 pH units	100/pkg	2601300
Pipet filler, safety bulb	each	1465100
Pipet, serological, 10-mL	each	53238
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet, volumetric, Class A, 15 mL	each	1451539
Silica Gel with indicator (for desiccator)	454 g	1426901

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Edition 7

Organic Carbon, Total

DOC316.53.01095

Direct Method[1] **Method 10128**

HR (100 to 700 mg/L C)

Scope and Application: For wastewater and industrial water

[1] U.S. Patent 6,368,870

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 260 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
A reagent blank is required for each series of samples.
To test for TOC levels below 100 mg/L C, use Method 10173 or Method 10129.

Collect the following items:

Description	Quantity
Total Organic Carbon Direct Method High Range Test 'N Tube™ Reagent Set	1
Cylinder, graduated, 10-mL	1
DRB200 Reactor	1
Light Shield or Adapter (see *Instrument-specific information*)	1
pH Paper	1
Flask, Erlenmeyer, 50-mL	1
Magnetic Stirrer	1
Pipet, TenSette®, 0.1 to 1.0 mL plus tips	1
Pipet, TenSette, 1.0 to 10.0 mL plus tips	1
Stir Bar, magnetic	1
Test Tube Rack	1

Collect the following items: (continued)

Description	Quantity
Water, organic-free	0.3 mL
Wipes, disposable	1

See *Consumables and replacement items* for reorder information.

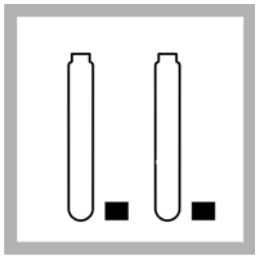
Direct method

1. Turn on the DRB200 reactor. Select the TOC program.

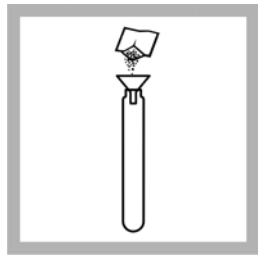
2. Use a graduated cylinder to add 10 mL of sample to a 50-mL Erlenmeyer flask that contains a stir bar.

3. Add 0.4 mL of Buffer Solution, pH 2.0. Use pH paper to make sure the sample pH is 2.

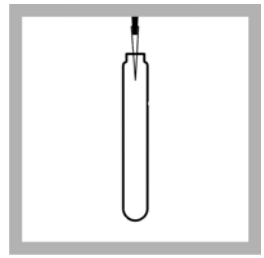
4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.



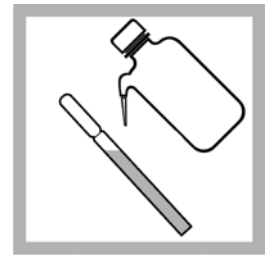
5. Label two High Range Acid Digestion vials *sample* and *reagent blank*.



6. Use a funnel to add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).

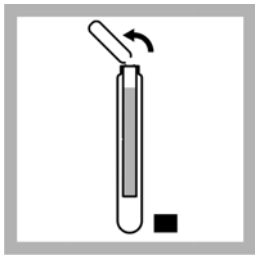


7. Use a TenSette® Pipet to add 0.3 mL of organic-free water to the reagent blank vial and 0.3 mL of prepared sample to the sample vial. Swirl to mix.



8. Rinse two blue MR/HR Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe. **Do not touch the ampules sides after wiping. Pick them up by the top.**

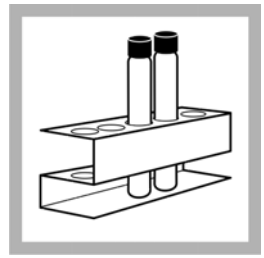
Direct method (continued)



9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, break the top off the ampule and allow it to drop into the Acid Digestion vial.

Do not invert or tilt the vial after inserting the ampule.

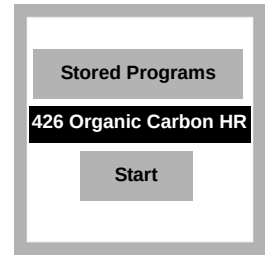
10. Cap the vial assemblies tightly, insert them in the reactor and close the lid for 2 hours at 103–105 °C.



11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for one hour for accurate results.

The liquid in the reagent blank vial should be dark blue.

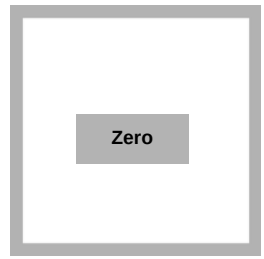


12. Select the test.

Insert a light shield or an adapter if required (see *Instrument-specific information*).

13. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.

14.



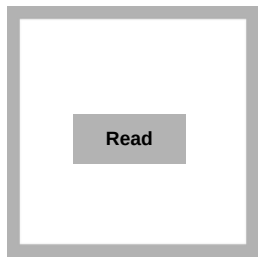
15. **ZERO** the instrument.

The display will show:

0 mg/L C

16. Wipe the sample vial assembly with a damp towel, followed by a dry one, to remove fingerprints or other marks.

17. Insert the sample vial assembly in the 16-mm round cell.



18. **READ** the results in mg/L C.

Interferences

The *Interfering substances* table lists substances that have been tested for interference and found not to interfere up to the levels indicated.

If the sample contains greater than 1000 mg/L $CaCO_3$ alkalinity, lower the sample pH to less than 7 before testing by adding Sulfuric Acid Solution.

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 50 NTU have been tested without interference.

Table 261 Interfering substances

Interfering substance	Interference level
Aluminum	10 mg/L Al
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br^-
Bromine	25 mg/L Br_2
Calcium	2000 mg/L as $CaCO_3$
Chloride	5000 mg/L Cl^-
Chlorine	10 mg/L Cl_2
Chlorine Dioxide	6 mg/L ClO_2
Copper	10 mg/L Cu
Cyanide	10 mg/L CN^-
Iodide	50 mg/L I^-
Iron (II)	10 mg/L Fe^{2+}
Iron (III)	10 mg/L Fe^{3+}
Magnesium	2000 mg/L as $CaCO_3$
Manganese (VII)	1 mg/L Mn
Monochloramine	14 mg/L NH_2Cl as Cl_2
Nitrite	500 mg/L NO_2^-
Ozone	2 mg/L O_3
Phosphate	3390 mg/L PO_4^{3-}
Silica	100 mg/L SiO_2
Sulfate	5000 mg/L SO_4^{2-}
Sulfide	20 mg/L S^{2-}
Sulfite	50 mg/L SO_3^{2-}
Zinc	5 mg/L Zn

Sample collection, preservation and storage

- Collect samples in clean glass bottles.
- Rinse the sample bottle several times with the sample to be collected.
- Fill the bottle completely full before capping.
- Test samples as soon as possible.

- Acid preservation is not recommended.
- Homogenize samples containing solids to assure representative samples.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- TOC Standard Ampule, 1000 mg/L C
- Organic-free Reagent Water
- 1-L Class A volumetric flask
- TenSette® Pipet and tips
- Acid Digestion Vials (3)
- TOC Persulfate Powder Pillow (1)
- 50 mL Class A Volumentric flask
- 15 mL Volumetric Pipet and pipet filler

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare a 300 mg/L C standard solution as follows:
   a. Transfer 15.00 mL of the 1000-mg/L total organic carbon standard solution to a 50-mL volumetric flask.
   b. Dilute to the mark with Organic-free Reagent water. Insert the stopper and mix thoroughly. Prepare this solution daily.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of the prepared 300-mg/L standard to three Acid Digestion Vials.
6. Add the contents of one TOC Persulfate Powder Pillow to each vial.
7. Add 0.3 mL of sample to each vial. Swirl to mix.
8. Continue the test starting at step 8 of the *Direct method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
9. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- TOC Standard Ampule, 1000 mg/L C
- Organic-free Reagent Water
- 50 mL Class A volumetric flask

- 15 mL Volumetric pipet and pipet filler

1. Prepare a 300 mg/L C standard solution as follows:
   a. Transfer 15.00 mL of the prepared 1000-mg/L organic carbon stock standard solution to a 50-mL volumetric flask.
   b. Dilute to the mark with Organic-free Reagent water. Insert the stopper and mix thoroughly. Prepare this solution daily.
2. Use this solution in place of the sample. Follow the *Direct method* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

To test for TOC levels below 100 mg/L C, use Method 10173 or Method 10129.

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
426	350 mg/L C	337–363 mg/L C	4 mg/L C

Summary of method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The absorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample. Test results are measured at 598 and 430 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total Organic Carbon Direct Method High Range Test 'N Tube™ Reagent Set, includes:	—	50 vials	2760445
Acid Digestion Solution Vials, High Range TOC[1]	1	50/pkg	—
Buffer Solution, Sulfate[1],[2]	0.4 mL	25 mL	—
Funnel, micro	1	each	2584335
Indicator Ampules, MR/HR TOC[1]	1	10/pkg	—
TOC Persulfate Powder Pillows[1]	1	50/pkg	—
pH Paper	1	5/pkg	39133
Water, Organic-free	0.3 mL	500 mL	2641549

[1] Not sold separately.

[2] See alternate size below

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 10-mL	1	each	50838

Required apparatus

Description	Quantity	Unit	Catalog number
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Flask, Erlenmeyer, 50-mL	1	each	50541
Magnetic Stirrer	1	each	2881200
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	2	50/pkg	2185696
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 1970010	2	50/pkg	2199796
Stir Bar, magnetic	1	each	4531500
Test Tube Rack	1–3	each	1864100
Wipes, Disposable	1	280/pkg	2097000

Recommended standards

Description	Unit	Catalog number
TOC Standard Solution Ampule (KHP Standard, 1000-mg/L C)	5/pkg	2791505

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity	115 VAC	2936701
Buffer Solution, Sulfate, pH 2.0	500 mL	45249
Flask, volumetric class A, 50 mL	each	1457441
Flask, volumetric class A, 1000 mL	each	1457453
Pipet, Volumetric, 15 mL	each	1451539
Pipet Filler	each	1465100
Pipet Tips, for TenSette Pipet 1970010	1000/pkg	2185628
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
Potassium Acid Phthalate	500 g	31534
Sulfuric Acid Solution, 5.25 N	100 mL	244932
Weighing Paper, 76 x 76 mm	500/pkg	1473800

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Edition 7

Organic Carbon, Total

DOC316.53.01093

Direct Method[1] **Method 10129**

LR (0.3 to 20.0 mg/L C)

Scope and Application: For water, drinking water and wastewater

[1] U.S. Patent 6,368,870

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 262 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
A reagent blank is required for each series of samples.
To test for higher ranges of TOC use Method 100173 or method 10128.

Collect the following items:

Description	Quantity
Total Organic Carbon Direct Method Low Range Test 'N Tube™ Reagent Set	1
Cylinder, graduated, 10-mL	1
DRB200 Reactor	1
Light Shield (see *Instrument-specific information*)	1
pH Paper	1
Flask, Erlenmeyer, 50-mL	1
Magnetic Stirrer	1
Pipet, TenSette®, 0.1 to 1.0 mL plus tips	1

Collect the following items: (continued)

Description	Quantity
Pipet, TenSette, 1.0 to 10.0 mL plus tips	1
Stir Bar, magnetic	1
Test Tube Rack	1
Water, organic-free	3.0 mL
Wipes, disposable, Kimwipes®	1

See *Consumables and replacement items* for reorder information.

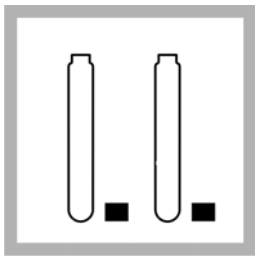
Direct method

1. Turn on the DRB200 reactor. Select the TOC program.

2. Use a graduated cylinder to add 10 mL of sample to a 50-mL Erlenmeyer flask that contains a stir bar.

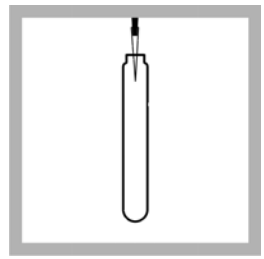
3. Add 0.4 mL of Buffer Solution, pH 2.0. Use pH paper to make sure the sample pH is 2.

4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.



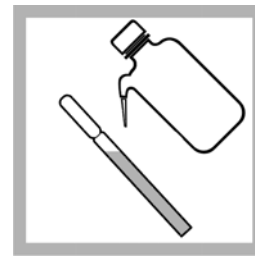
5. Label two Low Range Acid Digestion vials *sample* and *reagent blank*.

6. Use a funnel to add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).



7. Use a TenSette® Pipet to add 3.0 mL of organic-free water to the reagent blank vial and 3.0 mL of prepared sample to the sample vial. Swirl to mix.

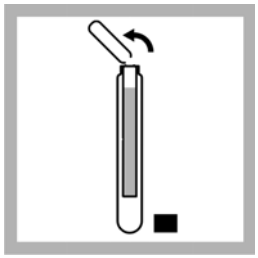
Note: *The organic free water must contain less than 0.05 mg/L carbon.*



8. Rinse two blue Low Range Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe.

Do not touch the ampule sides after wiping. Pick them up by the top.

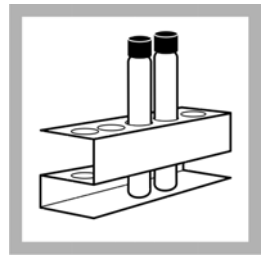
Direct method (continued)



9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Do not invert or tilt the vial after inserting the ampule.

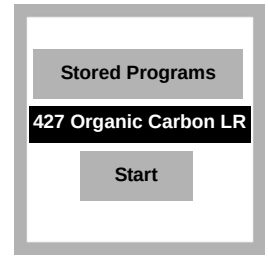
10. Cap the vial assemblies tightly, insert them in the reactor and close the lid for 2 hours at 103–105 °C.



11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for one hour for accurate results.

The liquid in the reagent blank vial should be dark blue.

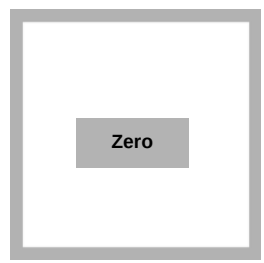


12. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

13. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.

14. Insert the reagent blank vial assembly in the 16-mm round cell holder.



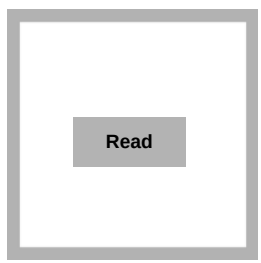
15. **ZERO** the instrument.

The display will show:

0.0 mg/L C

16. Wipe the sample vial assembly with a damp towel, followed by a dry one, to remove fingerprints or other marks.

17. Insert the sample vial assembly in the 16-mm round cell.



18. **READ** the results in mg/L C.

Interferences

The *Interfering substances* table lists substances that have been tested for interference and found not to interfere up to the levels indicated.

If the sample contains greater than 600 mg/L $CaCO_3$ alkalinity, lower the sample pH to less than 7 before testing by adding Sulfuric Acid Solution.

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 50 NTU have been tested without interference.

Table 263 Interfering substances

Interfering substance	Interference level
Aluminum	10 mg/L Al
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br^-
Bromine	25 mg/L Br_2
Calcium	2000 mg/L as $CaCO_3$
Chloride	500 mg/L Cl^-
Chlorine	10 mg/L Cl_2
Chlorine Dioxide	6 mg/L ClO_2
Copper	10 mg/L Cu
Cyanide	10 mg/L CN^-
Iodide	50 mg/L I^-
Iron (II)	10 mg/L Fe^{2+}
Iron (III)	10 mg/L Fe^{3+}
Magnesium	2000 mg/L as $CaCO_3$
Manganese (VII)	1 mg/L Mn
Monochloramine	14 mg/L NH_2Cl as Cl_2
Nitrite	500 mg/L NO_2^-
Ozone	2 mg/L O_3
Phosphate	3390 mg/L PO_4^{3-}
Silica	100 mg/L SiO_2
Sulfate	5000 mg/L SO_4^{2-}
Sulfide	20 mg/L S^{2-}
Sulfite	50 mg/L SO_3^{2-}
Zinc	5 mg/L Zn

Sample collection, preservation and storage

- Collect samples in clean glass bottles.
- Rinse the sample bottle several times with the sample to be collected.
- Fill the bottle completely full before capping.
- Test samples as soon as possible.
- Acid preservation is not recommended.
- Homogenize samples containing solids to assure representative samples.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- TOC Standard Ampule, 1000 mg/L C
- 100-mL Class A volumetric flask
- 15 mL Volumetric pipet and pipet filler
- Organic-free water
- TenSette® Pipet and tips
- Acid Digestion Vials (3)
- TOC Persulfate Powder Pillow (1)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare a 150-g/L C standard:
   a. Transfer 15.00 mL of 1000-mg/L TOC standard solution to a 100-mL Class A volumetric flask.
   b. Dilute to volume with organic-free water. Mix.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of the prepared standard to three Acid Digestion Vials.
6. Add the contents of one TOC Persulfate Powder Pillow to each vial.
7. Add 3.0 mL of sample to each vial. Swirl to mix.
8. Continue the test starting at step 8 of the *Direct method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
9. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- TOC Standard Ampule 1000 mg/L C
- Organic-free Reagent Water
- 10 mL Volumetric pipet and pipet filler
- 1-L Class A volumetric flask

1. Prepare a 10.0 mg/L C standard solution as follows:
   a. Transfer 10.00 mL of the 1000-mg/L total organic carbon standard solution to a 1000-mL (1 liter) volumetric flask.
   b. Dilute to the mark with Organic-free Reagent water. Insert the stopper and mix thoroughly. Prepare this solution daily.
2. Use this solution in place of the sample. Follow the *Direct method* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Reagent blanks

Water used for the reagent blank must contain less than 0.05 mg/L carbon. If the organic-free water container is left open for extended periods, the water may absorb carbon dioxide (CO_2) from the atmosphere. To remove the dissolved CO_2 from the organic-free water, it is necessary to acid-sparge it (see steps 2–4 of the procedure).

Generally, water stored in plastic containers is not suitable for low-range TOC blanks. Water stored in plastic may leach organic compounds from the container walls. The leached organic compounds usually cannot be removed by acid sparging.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
427	10.0 mg/L C	9.1–10.9 mg/L C	0.2 mg/L C

Summary of method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The absorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample. Test results are measured at 598 and 430 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Reagent Set, Total Organic Carbon Direct Method Low Range Test 'N Tube™, includes:	—	50 vials	2760345
Acid Digestion Solution Vials, High Range TOC[1]	1	50/pkg	—
Buffer Solution, Sulfate[1],[2]	0.4 mL	25 mL	45233
Funnel, micro	1	each	2584335
Indicator Ampule, Low Range TOC[1]	1	10/pkg	—
TOC Persulfate Powder Pillows[1]	1	50/pkg	—
pH Paper	1	5/pkg	39133
Water, Organic-free	3.0 mL	500 mL	2641549

[1] Not sold separately.

[2] See alternate size below

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 10-mL	1	each	50838
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Flask, Erlenmeyer, 50-mL	1	each	50541
Magnetic Stirrer	1	each	2881200
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	2	50/pkg	2185696
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 1970010	2	50/pkg	2199796
Stir Bar, magnetic	1	each	4531500
Test Tube Rack	1	each	1864100
Wipes, Disposable	1	280/pkg	2097000

Recommended standards

Description	Unit	Catalog number
TOC Standard Solution Ampule (KHP Standard, 1000-mg/L C)	5/pkg	2791505

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity	115 VAC	2936701
Buffer Solution, Sulfate pH 2.0	500 mL	45249
Flask, volumetric class A, 1000 mL	each	1457453

Optional reagents and apparatus

Description	Unit	Catalog number
Flask, volumetric class A, 100 mL	each	1457442
Pipet filler	each	1465100
Pipet Tips, for TenSette Pipet 19700–10	1000/pkg	2185628
Pipet Tips, for TenSette Pipet 19700–10	250/pkg	2199725
Pipet, Volumetric, Class A, 15 mL	each	1451539
Pipet, Volumetric, Class A, 10 mL	each	1451538
Potassium Acid Phthalate	500 g	31534
Sulfuric Acid Solution, 5.25 N	100 mL	244932
Weighing Paper, 76 x 76 mm	500/pkg	1473800

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Edition 7

Organic Carbon, Total

DOC316.53.01094

Direct Method[1] **Method 10173**

MR (15 to 150 mg/L C)

Scope and Application: For water, drinking water and wastewater

[1] U.S. Patent 6,368,870

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 264 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
A reagent blank is required for each series of samples.
To test for higher ranges of TOC use Method 10128. To test lower ranges of TOC use method 10129.

Collect the following items:

Description	Quantity
Total Organic Carbon Direct Method Mid Range Test 'N Tube™ Reagent Set	1
Cylinder, graduated, 10-mL	1
DRB200 Reactor	1
Light Shield or Adapter (see *Instrument-specific information*)	1
pH Paper	1
Flask, Erlenmeyer, 50-mL	1
Magnetic Stirrer	1
Pipet, TenSette®, 0.1 to 1.0 mL plus tips	1

Collect the following items: (continued)

Description	Quantity
Pipet, TenSette, 1.0 to 10.0 mL plus tips	1
Stir Bar, magnetic	1
Test Tube Rack	1
Water, organic-free	3.0 mL
Wipes, disposable	1

See *Consumables and replacement items* for reorder information.

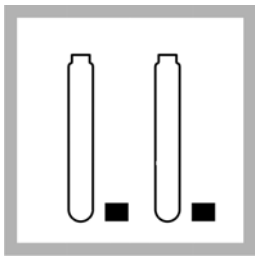
Direct method

1. Turn on the DRB200 reactor. Select the TOC program.

2. Use a graduated cylinder to add 10 mL of sample to a 50-mL Erlenmeyer flask that contains a stir bar.

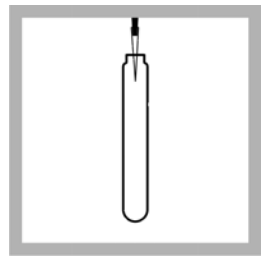
3. Add 0.4 mL of Buffer Solution, pH 2.0. Use pH paper to make sure the sample pH is 2.

4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.

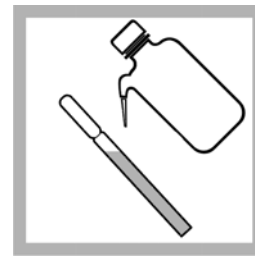


5. Label two Mid Range Acid Digestion vials *sample* and *reagent blank*.

6. Use a funnel to add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).



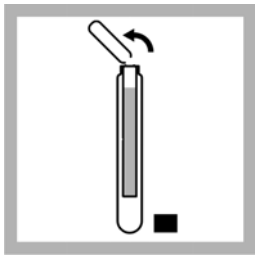
7. Use a TenSette® Pipet to add 1.0 mL of organic-free water to the reagent blank vial and 1.0 mL of prepared sample to the sample vial. Swirl to mix.



8. Rinse two blue MR/HR Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe.

Do not touch the ampule sides after wiping. Pick them up by the top.

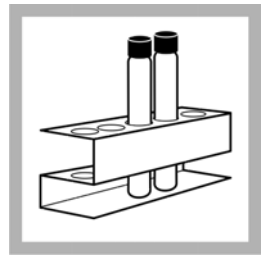
Direct method (continued)



9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Do not invert or tilt the vial after inserting the ampule.

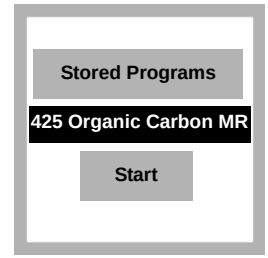
10. Cap the vial assemblies tightly, insert them in the reactor and close the lid for 2 hours at 103–105 °C.



11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for one hour for accurate results.

The liquid in the reagent blank vial should be dark blue.

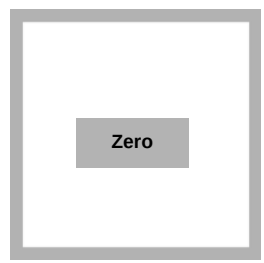


12. Select the test.

Insert a light shield or an adapter if required (see *Instrument-specific information*).

13. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.

14.



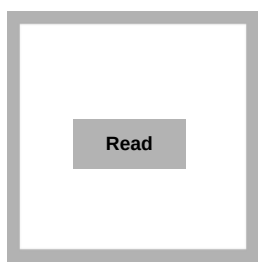
15. **ZERO** the instrument.

The display will show

0 mg/L C.

16. Wipe the sample vial assembly with a damp towel, followed by a dry one, to remove fingerprints or other marks.

17. Insert the sample vial assembly in the 16-mm round cell.



18. **READ** the results in mg/L C.

Interferences

The *Interfering substances* table lists substance that have been tested for interference and found not to interfere up to the levels indicated.

If the sample contains greater than 1000 mg/L $CaCO_3$ alkalinity, lower the sample pH to less than 7 before testing by adding Sulfuric Acid Solution.

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 50 NTU have been tested without interference.

Table 265 Interfering substances

Interfering substance	Interference level
Aluminum	10 mg/L Al
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br^-
Bromine	25 mg/L Br_2
Calcium	2000 mg/L as $CaCO_3$
Chloride	1500 mg/L Cl^-
Chlorine	10 mg/L Cl_2
Chlorine Dioxide	6 mg/L ClO_2
Copper	10 mg/L Cu
Cyanide	10 mg/L CN^-
Iodide	50 mg/L I^-
Iron (II)	10 mg/L Fe^{2+}
Iron (III)	10 mg/L Fe^{3+}
Magnesium	2000 mg/L as $CaCO_3$
Manganese (VII)	1 mg/L Mn
Monochloramine	14 mg/L NH_2Cl as Cl_2
Nitrite	500 mg/L NO_2^-
Ozone	2 mg/L O_3
Phosphate	3390 mg/L PO_4^{3-}
Silica	100 mg/L SiO_2
Sulfate	5000 mg/L SO_4^{2-}
Sulfide	20 mg/L S^{2-}
Sulfite	50 mg/L SO_3^{2-}
Zinc	5 mg/L Zn

Sample collection, preservation and storage

- Collect samples in clean glass bottles.
- Rinse the sample bottle several times with the sample to be collected.
- Fill the bottle completely full before capping.
- Test samples as soon as possible.
- Acid preservation is not recommended.

- Homogenize samples containing solids to assure representative samples.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- 1000-mg/L C TOC Standard Ampule
- 50-mL Class A volumetric flask
- 15 mL Volumetric pipet and pipet filler
- Organic-free water
- TenSette® Pipet and tips
- Acid Digestion Vials (3)
- TOC Persulfate Powder Pillow (1)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare a 300-mg/L C standard:
   a. Transfer 15.00 mL of 1000-mg/L TOC standard solution to a 50-mL Class A volumetric flask.
   b. Dilute to volume with organic-free water. Insert the stopper and mix thoroughly.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of the 300-mg/L standard to three Acid Digestion Vials.
6. Add the contents of one TOC Persulfate Powder Pillow to each vial.
7. Add 1.0 mL of sample to each vial. Swirl to mix.
8. Continue the test starting at step 8 of the *Direct method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
9. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- TOC standard Ampule, 1000 mg/L C
- Organic-free Reagent Water
- 5 mL Volumetric pipet and pipet filler
- 50-mL Class A volumetric flask

1. Prepare a 100 mg/L C standard solution as follows:
   a. Transfer 5.00 mL of the 1000-mg/L total organic carbon standard solution to a 50-mL volumetric flask.
   b. Dilute to volume with Organic-free Reagent water. Insert the stopper and mix thoroughly. Prepare this solution daily.
2. Use this solution in place of the sample. Follow the *Direct method* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
425	DR 5000	70 mg/L C	68–72 mg/L C	1.2 mg/L C

Summary of method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The absorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample. Test results are measured at 598 and 430 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Reagent Set, Total Organic Carbon Direct Method Mid Range Test 'N Tube™, includes:	—	50 vials	2815945
Acid Digestion Solution Vials, High Range TOC[1]	1	50/pkg	—
Buffer Solution, Sulfate[1,2]	0.4 mL	25 mL	—
Funnel, micro	1	each	2584335
Indicator Ampules, MR/HR TOC[1]	1	10/pkg	—
TOC Persulfate Powder Pillows[1]	1	50/pkg	—

Required reagents

Description	Quantity/Test	Unit	Catalog number
pH Paper	1	5/pkg	39133
Water, Organic-free	1.0 mL	500 mL	2641549

[1] Not sold separately.

[2] See alternate size below

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 10-mL	1	each	50838
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Flask, Erlenmeyer, 50-mL	1	each	50541
Magnetic Stirrer	1	each	2881200
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 1970001	2	50/pkg	2185696
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 1970010	2	50/pkg	2199796
Stir Bar, magnetic	1	each	4531500
Test Tube Rack	1	each	1864100
Wipes, Disposable	1	280/pkg	2097000

Recommended standards

Description	Unit	Catalog number
TOC Standard Solution Ampule, 20 mL (KHP Standard, 1000-mg/L C)	5/pkg	2791505

Optional reagents and apparatus

Description	Unit	Catalog number
Sulfuric Acid Solution, 5.25 N	100 mL-MDB	244932
Pipet Tips, for TenSette Pipet 19700–10	1000/pkg	2185628
Pipet Tips, for TenSette Pipet 19700–10	250/pkg	2199725
Balance, analytical, 80 g capacity	115 VAC	2936701
Weighing Paper, 76 x 76 mm	500/pkg	1473800
Flask, volumetric class A, 50 mL	each	1457441
Flask, volumetric class A, 1000 mL	each	1457453
Potassium Acid Phthalate	500 g	31534
Buffer solution, sulfate, pH 2.0	500 mL	45249
Pipet, Volumetric, Class A, 15 mL	each	1451539
Pipet, Volumetric, Class A, 5 mL	each	1451537
Pipet Filler	each	1465100

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Edition 7

Organic Constituents UV Absorbing (UV-254)

DOC316.53.01092

Direct Reading Method[1] — Method 10054

Scope and Application: To indicate the total concentration of UV-absorbing organic compounds in drinking water and drinking water source waters.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*, Method 5910.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 266 Instrument-specific information

Instrument	Sample cell	Cell orientation	Adapter
DR 6000	2624410	Align clear windows facing right/left	—
DR 5000	2624410	Align clear windows facing the user	Multi-cell Adapter, 10-mm faces user

Before starting the test:

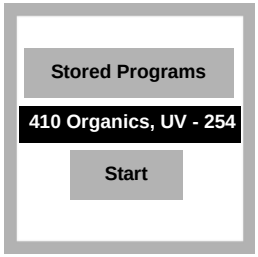
The sample pH should be between 4 and 10. If not, see *Interferences* in this procedure. Samples used for SUVA calculations **must not** be pH adjusted
Any non-plastic filter assembly can be used for this test. Use a 0.45-µm or glass fiber filter of nominal pore size (1–1.5 µm) without organic binder. A 0.45-µm filter must be utilized if the results are to be used for SUVA calculations.
Handle the cell on the frosted sides only.
Use only Organic-Free Reagent Water to zero the instrument.
The Chromic Acid Cleaning Solution is regulated as a hazardous waste for chromium (D007) and corrosivity (D002) when disposed per Federal RCRA. Refer to the current MSDS for safe handling and disposal instructions.

Collect the following items:

Description	Quantity
Organic-free Reagent Water	varies
Filter Assembly	1
Stand, buret	1
Sample Cell (see *Instrument-specific information*)	1

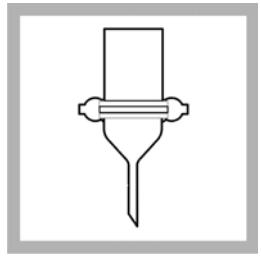
See *Consumables and replacement items* for reorder information.

Organic Constituents

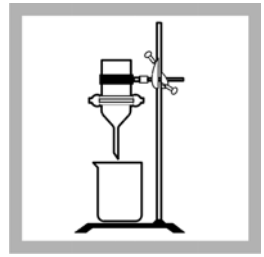


1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Assemble the filter apparatus. Be sure to use the white PTFE support plate. Insert the filter with the wrinkled surface upward.

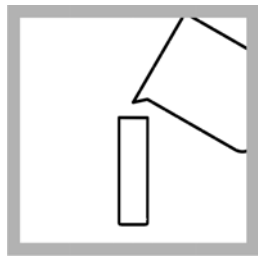


3. Mount the apparatus into a support stand and place a clean glass beaker underneath.

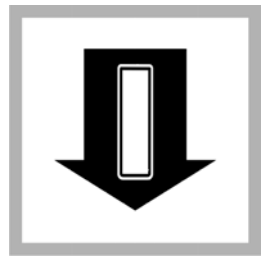
4. Prewash the filter assembly by pouring at least 50 mL of Organic-Free Reagent Water through the filter. Discard the filtered water.

Pre-rinsing removes any soluble impurities from the filter.

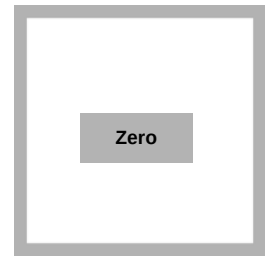
5. Prepared Sample: Pour 50 mL of sample through the filter and collect the filtered sample.



6. Blank Preparation: Rinse a clean 1-cm quartz cell several times with Organic-Free Reagent Water. Fill the cell with Organic-Free Reagent Water. Wipe the cell walls thoroughly.



7. Align the clear windows with the light path. Insert the blank into the cell holder.



8. **ZERO** the instrument.

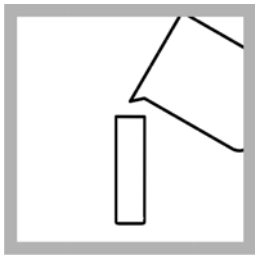
The display will show:

0.000 cm^{-1}

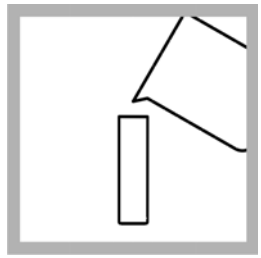
1-cm cell

Lamp Warm Up will be indicated if the UV lamp has not been previously on. This may take 2–3 minutes.

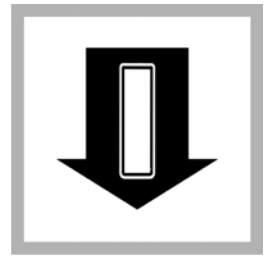
Organic Constituents (continued)



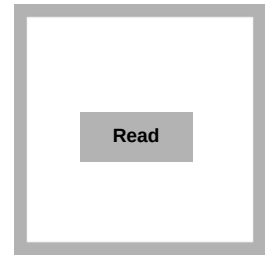
9. Discard the contents of the blank cell and rinse the cell several times with filtered sample.



10. After rinsing, fill the cell with filtered sample. Wipe the cell walls to remove fingerprints.



11. Align the clear windows with the light path. Insert the cell containing the prepared sample into the cell holder.



12. **READ** the results in absorbance per centimeter (cm^{-1}).

For optimum results, the cm^{-1} value should fall between 0.005 and 0.900. If the value is less than 0.005 absorbance using a 1-cm cell, use a 5-cm or 10-cm quartz cell.

To test with the 5-cm or 10-cm cell:

1. Press **OPTIONS>MORE>CHEMICAL FORMS**.
2. Press **5 CM** or **10 CM.** Press **OK>RETURN**.
3. The displayed results (in absorbance per centimeter) will be corrected for the 5-cm or 10-cm cell pathlength selected. If cm^{-1} results are greater than 0.900, accurately dilute the sample with Organic-Free Reagent Water. Correct the test result by the appropriate dilution factor.

Interferences

Table 267 Interfering substances

Interfering substance	Interference level
Sample pH outside 4–10	Add either 1 N Sodium Hydroxide or 1 N Sulfuric Acid to the sample to adjust sample between pH 4-10.
UV-absorbing inorganics (bromide, ferrous iron, nitrate, nitrites)	Follow the UV scanning procedure below.
UV-absorbing Oxidants and reductants (chloramines, chlorates, chlorites, ozone, thiosulfates)	Follow the UV scanning procedure below.

To determine the presence of interferences, a scan of the filtered sample versus Organic-Free Reagent Water is recommended on a regular basis:

1. From the main menu, press **WAVELENGTH SCAN>OPTIONS>**λ.
2. Press **200>OK**.
3. Press **400>OK**.
4. Press **1 NM>OK**.

5. Insert the cell containing the Organic-Free Reagent Water (the blank) into the cell compartment.

6. Press **ZERO**. The baseline scan from 200 to 400 nm will begin.

Note: *Lamp Warm Up will be indicated if the UV lamp has not been previously on. This may take 2–3 minutes.*

7. After the baseline scan is recorded, insert the cell containing the filtered sample into the cell compartment. Press **READ** to scan the sample.

If the sample scan shows relatively sharp peaks, interferences may be present. Generally, natural organic matter will show a relatively featureless curve in the UV region with increasing absorption as the wavelength decreases. If the sharp peaks are indicated, an alternate wavelength should be selected and reported.

Sample collection, preservation and storage

- Collect samples in cleaned glass containers. Do not use plastic containers.
- Analyze samples as soon as possible after collection.

Cell cleaning

New or dirty cells should be soaked with Chromic Acid Cleaning Solution to remove trace organic contamination.

1. Allow to soak overnight or up to 12 hours.

2. After soaking, rinse with at least 10 volumes of Organic-Free Reagent Water.

Treatment of cells with chromic acid is required only occasionally if cells are rinsed with Organic-Free Reagent Water after use.

Method performance

Standard: There is no primary standard or calibration for the UV-254 method. Using a Potassium Acid Phthalate solution equivalent to 30-mg/L as carbon, the following reproducibility data was obtained using one instrument. Refer to Standard Methods for the standard preparation.

Program	Precision—95% Confidence Limits of Distribution
410	0.431 - 0.433 cm^{-1}

Summary of method

Filtered sample is measured at 254 nm against organic-free water as a indicator of organic constituents in the sample water. Results are automatically reported in absorbance per centimeter (cm^{-1}). The results can be used in calculating Specific Ultraviolet Absorbance (SUVA).

Estimated detection limit

Because this test is a non-specific measurement for organic constituents, there is no estimated detection limit for program 410.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Organic-Free Reagent Water	varies	500 mL	2641549

Required apparatus

Description	Unit	Catalog number
Beaker, 100-mL	each	50042H
Buret Stand	each	32900
Clamp Holder	each	32600
Clamp, 3-Prong	each	42200
Filter Funnel Assembly, 7-cm	each	2164100
Filter Plate, PTFE, for 21641-00	each	2164200
Filter, glass fiber, 70-mm	100/pkg	253053
Sample Cell, quartz, 1-cm (10 mm)	each	2624410
Sulfuric Acid, 1 N	100 mL MDB	127032

Optional reagents

Description	Unit	Catalog number
Chromic Acid Cleaning Solution	500 mL	123349
Sodium Hydroxide Standard Solution, 1.00 N	100 mL MDB[1]	104532

[1] Larger sizes are available.

Optional apparatus

Description	Unit	Catalog number
Cell Holder for 10-cm (100 mm) sample cells (DR 5000 only)	each	LZY421
Cylinder, graduated, 50-mL	each	50841
Filter, membrane, 47-mm; 0.45-microns, hydrophilic, polyethersulfone SUVA	each	2894700
Filter Holder, glass for vacuum filtration (SUVA)	each	234000
Flask, filtering, glass, 1000-mL (SUVA)	each	54653
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	39133
Tubing, Latex rubber (SUVA)	12 ft	56019
Potassium Acid Phthalate	500 g	31534
Sample Cell, quartz, 1-cm (10 mm), matched pair	pair	4822800
Sample Cell, quartz, 5-cm (50 mm)	each	2624450
Sample Cell, quartz, 10-cm (100 mm)	each	2624401
Standard Methods Book, most current edition	1	2270800
Aspirator (SUVA)	1	213100

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Edition 7

Oxygen Demand, Biochemical

DOC316.53.01200

Dilution Method[1]

Method 8043

Scope and Application: For water and wastewater.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater* and from Klein, R.L.; Gibbs, C. *Journal of Water Pollution Control Federation*, 1979, 51(9), 2257.

Test preparation

Before starting the test:

The BOD test is a 5-day test. Follow all steps carefully to make sure that the test does not have to be repeated.
The dilution water for this test must not have an oxygen demand or any toxins. When incubated for 5 days at 20 °C, the dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L.
Carbonaceous BOD (CBOD) can be determined by the addition of nitrification inhibitor. A test for CBOD is recommended for biologically treated effluents, samples seeded with biologically treated effluents and river water.
The *Troubleshooting—Graphical calculation method* provides an alternate system for calculating results and is a convenient tool for troubleshooting problems in BOD measurements. The graphical calculation method is not approved for regulatory reporting.

Collect the following items:

Description	Quantity
BOD bottles, 300-mL, glass, with glass stoppers and plastic caps	6
Dilution water containing nutrient buffer and seed (see *Dilution water preparation*)	varies
Nitrification inhibitor (for CBOD only)	1 bottle
Pipet, serological	1
Incubator	1

See *Consumables and replacement items* for reorder information.

Dilution method

1. Prepare the dilution water using a BOD Nutrient Buffer Pillow. See *Dilution water preparation*.

2. Select the sample volumes. See *Sample size selection*.

Note: *If the minimum sample volume is 3 mL or more, determine the dissolved oxygen in the undiluted sample; this determination can be omitted when analysing sewage and settled effluents known to have a dissolved oxygen content near 0 mg/L.*

When analyzing disinfected samples or industrial effluents, refer to *Interferences*.

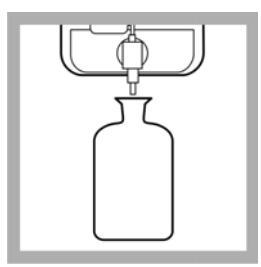
3. Stir the sample gently with the pipet. Use the pipet to add the minimum sample volume to the first BOD bottle.

Add the remaining four sample volumes to four more BOD bottles. Mark the bottles and record the contents of each bottle.

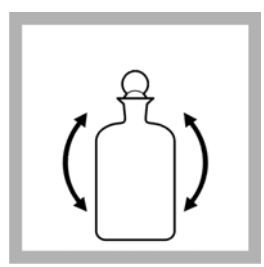
4. Fill an additional BOD bottle with dilution water only. This will be the dilution water blank.

5. If the test is for CBOD, add two portions of Nitrification Inhibitor (approximately 0.16 g) to each bottle.

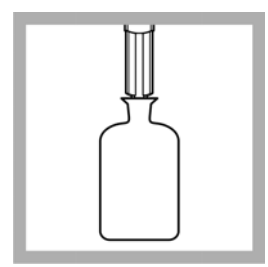
The oxidation of nitrogen compounds will be prevented. Report results as CBOD.



6. Fill each bottle to just below the lip with dilution water. Allow the dilution water to flow down the sides of the bottle to prevent air bubbles from becoming trapped in the bottle.



7. Stopper the bottles carefully to prevent air bubbles from becoming trapped. Tightly twist the stopper into place. Press down on the stopper and invert the bottles several times to mix.



8. Measure the initial dissolved oxygen concentration in each bottle. Use a probe and meter or titration. If a titration is used, two sets of BOD bottles must be prepared.

Be sure to measure the DO of the dilution water blank.

Dilution method

9. Stopper the bottles carefully to prevent air bubbles from becoming trapped. Add dilution water to the lip of each BOD bottle to make a water seal.

10. Place a plastic cap over the lip of each bottle. Put the bottles in an incubator at 20 (±1) °C. Incubate for five days.

11. After five days, measure the remaining dissolved oxygen concentration in each bottle.

At least 1.0 mg/L DO should be left in each BOD bottle.

12. Calculate the BOD value (see *Calculation Methods—Standard Methods*).

Dilution water preparation

The dilution water must be prepared very carefully to make sure that no source of oxygen demand or toxins are added. The water that is used to prepare the dilution water must be of very high quality. The water must not have any organic compounds or any toxic compounds such as chlorine, copper and mercury.

Use the following guidelines to make sure the dilution water is of high quality.

Guidelines

- Use distilled water from an alkaline permanganate distillation for the best results.
- Do not use deionized water from ion exchange columns. The resins in the cartridges (especially new cartridges) will occasionally release organic materials that have an oxygen demand. In addition, bacteria can grow on the columns and contaminate the dilution water.
- Store the distilled water in clean jugs in an incubator at 20 °C. Fill containers till about ¾ full and shake the jugs to saturate the water with air, or cap the jugs loosely and store for 24 hours or more, to allow dissolution of oxygen.
- A small aquarium pump or air compressor can be used to saturate the water with air. Make sure that the air is filtered and that the filter does not grow bacteria. Clean the apparatus before and after use.
- Add the nutrients and seed (if necessary) to the distilled water immediately before the test.
- The dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L when incubated for 5 days at 20 °C.

Procedure

1. Prepare and store the distilled water at 20 °C (see *Guidelines*).
2. Select a BOD nutrient buffer pillow from the *BOD nutrient buffer pillows* table.
3. Tap the pillow on a hard surface then shake the pillow to mix the contents.

4. Add the contents of the pillow to the distilled water in a jug with ample headspace above the water. Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.

5. If the sample is known to be low in bacteria, for example industrial waste or sewage that has been disinfected, add 3 mL of bacterial seed to each liter of the dilution water. Use raw sewage for the bacterial seed. Allow the sewage to stand undisturbed at 20 °C for 24 to 36 hours before use. Pipet from the upper portion of the sewage. Make sure to measure the BOD of the seed so that it can be subtracted from the BOD of the sample. A seed that has a BOD of 200 mg/L (a typical range for domestic sewage) will typically deplete at least 0.6 mg/L DO, when added at a rate of 3 mL/L of dilution water. If insufficient oxygen depletion occurs, increase the quantity of the seed.

Table 268 BOD nutrient buffer pillows

Volume of dilution water to prepare	BOD nutrient buffer pillow catalog no.
300 mL (add pillow to each BOD bottle)	1416066
3 liters	1486166
4 liters	2436466
6 liters	1486266
19 liters	1486398

Note: *To prepare dilution water by the conventional method, pipet 1 mL of each of the following solutions per liter of distilled water at 20 °C: Calcium Chloride Solution, Ferric Chloride Solution, Magnesium Sulfate Solution, and Phosphate Buffer Solution. Cap the bottle and shake vigorously for one minute. The Phosphate Buffer Solution should be refrigerated to decrease the rate of biological growth. Use care with all solutions to avoid contamination.*

Sample size selection

Make an estimation of the sample volumes that are necessary for the test. At least 2.0 mg/L of dissolved oxygen (DO) should be consumed during the test and at least 1.0 mg/L DO should be left in the BOD bottle.

Samples such as raw sewage will have a high BOD. Small sample volumes must be used because large samples will consume all of the oxygen. Samples with a low BOD must use larger sample volumes to make sure that enough oxygen is consumed to give accurate results.

The elevation of the laboratory changes the amount of oxygen that can dissolve in water (see *Oxygen saturation values at various altitudes (20 °C)*). At higher elevations, the amount of oxygen that can dissolve in water decreases, so less oxygen is available to microorganisms.

Procedure

1. Refer to the *Minimum sample volume* table to select the minimum sample volume. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the minimum sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the minimum sample volume is 15 mL.

2. Refer to the *Maximum sample volume* table to select the maximum sample volume. At 1000 feet, with an estimated BOD of 300 mg/L, the largest sample volume is 8 mL. For a BOD of 40 mg/L the maximum volume is 60 mL (also at 1000 feet).

3. Select two or more other sample volumes between the minimum and maximum volumes so that there are four or five sample volumes total.

Table 269 Minimum sample volume

Sample type	Estimated BOD (mg/L)	Minimum sample volume (mL)
Strong trade waste	600	1
Raw and settled sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted river waters	6	100
	4	200
	2	300

Table 270 Maximum sample volume[1]

BOD at sea level	BOD at 1000 ft	BOD at 5000 ft	Maximum sample volume (mL)
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

[1] Samples with higher concentrations should be pre-diluted, per Standard Methods.

Table 271 Oxygen saturation values at various altitudes (20 °C)

Altitude (ft)	Average Pressure in mBar at this altitude	Oxygen value (mg/L) in water saturated with air
Sea level (0)	1013	9.09
1000	977	8.76
2000	942	8.44
3000	908	8.13
4000	875	7.82
5000	843	7.53
6000	812	7.24

Calculation Methods—Standard Methods

Use the Standard Methods calculation when the results must be reported to a regulatory agency.

When dilution water is not seeded:

$$BOD_5,\ mg/L = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$BOD_5,\ mg/L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where:

BOD_5 = BOD value from the 5-day test

D^1 = DO of diluted sample immediately after preparation, in mg/L

D^2 = DO of diluted sample after 5 day incubation at 20 °C, in mg/L

P = Decimal volumetric fraction of sample used

B^1 = DO of seed control before incubation, in mg/L

B^2 = DO of seed control after incubation, in mg/L

f = ratio of seed in diluted sample to seed in seed control =

(% seed in diluted sample)/(% seed in seed control) OR

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification inhibitor was added.

Averaged results are acceptable if more than one sample dilution meets all of the following criteria:

- The remaining DO is at least 1 mg/L
- The final DO value is at least 2 mg/L lower than the initial DO value
- There is no evidence of toxicity at higher sample concentrations
- There are no obvious anomalies

Interferences

Many chlorinated and industrial effluents require special handling to ensure reliable BOD results. Usually, careful experimentation with the particular sample will indicate what modifications should be made to the test procedure.

Toxins in the sample will adversely affect any microorganisms present and result in lower BODs.

To eliminate small amounts of residual chlorine, allow the sample to stand for one to two hours at room temperature. For larger quantities, determine the amount of sodium thiosulfate to add to the sample as follows:

c. Measure 100 mL of sample into a 250-mL Erlenmeyer flask. Using a 10-mL serological pipet and a pipet filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100-g/L, to the flask.

d. Add three full droppers of Starch Indicator Solution and swirl to mix.

e. Fill a 25-mL buret with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.

f. Calculate the amount of 0.025 N Sodium Thiosulfate Standard Solution to add to the sample:

$$\text{mL 0.025 N sodium thiosulfate required} = \frac{\text{mL titrant used x volume of remaining sample}}{100}$$

g. Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample. Mix thoroughly. Wait 10 to 20 minutes before running the BOD test.

To eliminate the effect of phenols, heavy metals or cyanide, dilute the sample with high quality distilled water. Alternately, the seed used in the dilution water may be acclimatized to tolerate such materials. Acclimatize seed as follows:

a. Fill a one-gallon stainless steel or plastic container with domestic sewage and aerate for 24 hours. Allow the heavier material to settle.

b. After settling for one hour, siphon off three quarts of material and discard.

c. Fill the container with a mixture of 90% sewage and 10% wastes containing the toxic material.

d. Aerate for 24 hours. Repeat steps b and c with increasing amounts of waste until the container holds 100% toxic waste material.

Optimum pH for the BOD test is between 6.5 and 7.5. Adjust samples to pH 7.2 with Phosphate Buffer Solution or 1 N Sulfuric Acid or Sodium Hydroxide Standard Solution if the pH is not in this range.

Cold samples may be supersaturated with oxygen and will have low BOD results. Fill a one-quart bottle about halfway with cold sample and shake vigorously for two minutes. Allow sample to reach 20 °C. Then shake the bottle vigorously for two minutes.

Accuracy check

ezGGA Method
Required for accuracy check:

- BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL (300-mg/L of glucose and 300-mg/L of glutamic acid)
- Seeded dilution water
- 4 BOD bottles
- 1.0–4.0 mL Class A volumetric pipets and pipet filler or 1–10 mL TenSette Pipet and Pipet tips
- Dissolved oxygen measurement apparatus

DO measurement with the LBOD probe:

1. Add the necessary seed to a 300-mL BOD bottle.
2. Fill the BOD bottle with dilution water until the water level is approximately ¼ inch up the ground glass portion of the neck. (See dimension “x” in illustration).
3. Put the 2-mL BOD standard ampule into the ampule breaker and rinse the assembly with deionized water.
4. Hold the ampule and breaker over the rim of the BOD bottle.
5. Use the ampule breaker to open the ampule and allow it to fall into the BOD bottle. Leave ampule in the BOD bottle during incubation period.
6. Follow the general procedure for the BOD test.
7. Calculate the BOD concentration of the standard solution. The 2 mL in the vial is equivalent to 6 mL as prepared by Standard Methods. Calculate the BOD concentration as though there were 6 mL added to the bottle instead of 2 mL. The dilution factor for this standard is 50x.

DO measurement with the Clark Cell electrode:

1. Add the necessary seed to a 300-mL BOD bottle.
2. Use the ampule breaker to open the ampule.
3. Pour the contents of the ampule into the BOD bottle. Tap the ampule on the rim of the bottle to dislodge the contents. Do not drop ampule into the bottle when using a Clark Cell.
4. Fill the ampule with buffered dilution water and add the water to the BOD bottle.
5. Repeat step 4.
6. Fill the BOD bottle with dilution water until the water level is approximately ½ inch up the ground glass portion of the neck.
7. Follow the general procedure for the BOD test.
8. Calculate the BOD concentration of the standard solution. The 2 mL in the vial is equivalent to 6 mL as prepared by Standard Methods. Calculate the BOD concentration as though there were 6 mL added to the bottle instead of 2 mL. The dilution factor for this standard is 50x.

Note: *The ampules include precisely 2 mL of 450 mg/L GGA. Pouring the entire solution into the bottle is the same as adding 6 mL of 150 mg/L solution as per the Standard Methods.*

Troubleshooting—Graphical calculation method

The Graphical Method helps troubleshoot problems in BOD measurements. This method cannot be used when the results must be reported to a regulatory agency.

1. Plot the mg/L dissolved oxygen (DO) remaining in each diluted sample versus the mL sample taken. Draw the best straight line through the plotted points. See *Dissolved Oxygen per mL of Sample*.

Note: *An erroneous point is visually evident at this time and can be disregarded. However, at least three points should be on the line or very close to it. For unseeded dilution water, the line should cross the mg/L DO Remaining scale near or below the oxygen saturation value for the altitude of the laboratory as discussed in Dilution water preparation.*

2. To calculate the BOD, use the following equation which is mathematically equivalent to the BOD equation in Standard Methods.

   mg/L BOD = (A x 300) – B + C

   where:

   A = the slope

   The slope of the line is equal to the mg/L DO consumed per mL of sample taken. Take any point on the line and subtract the mg/L DO Remaining at that point from the mg/L DO where the line crosses the DO scale (Y intercept, mg/L DO Remaining). Divide the difference by the mL of sample at the point chosen.

   300 = the volume of the BOD bottle

   B = the Y intercept

   This is the DO value where the line crosses the “DO Remaining” scale. (This should be very close to the actual dilution water blank value.)

   C = the sample DO

   This is the DO of the undiluted sample.

   Another way to write this equation is:

   mg/L BOD = (Slope x 300) – Y intercept + Sample DO

   Note: *If the best straight line is obtained by linear regression through use of a calculator, the sign (-) of the slope must be changed (+) before multiplying by 300.*

Example:
The mg/L DO remaining was determined for a series of four dilutions of domestic sewage after five days of incubation. Results were as follows:

mL of sample taken	mg/L DO remaining
2.0	7.50
3.0	6.75
6.0	4.50
9.0	2.25

The DO values were plotted versus the mL of sample taken and a straight line drawn as in *Dissolved Oxygen per mL of Sample*. If a set of BOD dilutions is run correctly with a homogeneous sample, a graph of the mg/L DO remaining versus the sample volume would result in a straight line. The value where the line intersects the y-axis is equal to the DO content of the dilution water

after incubation, although this is not actually measured. In this case, it was equal to 9.0 mg/L and the DO of the domestic sewage sample was assumed to be zero. If another type of sample is used, the DO of an undiluted sample should be measured either by the Winkler titration or with a luminescent or electrochemical probe.

The *Calculation Methods—Standard Methods* formula for calculating BOD also can be written as follows (not approved for reporting purposes):

$$\frac{\text{mg/L DO remaining w/smaller sample volume} - \text{mg/L DO remaining w/larger sample volume}}{\text{mL of larger sample volume} - \text{mL of smaller sample volume}} \times 300 - DO_D + S = \text{mg/L BOD}$$

Using this information in the example:

mg/L DO remaining with smaller sample volume = 7.50

mg/L DO remaining with larger sample volume = 2.25

mL of larger sample volume = 9.0

mL of smaller sample volume = 2.0

300 = volume (mL) of BOD bottle

DO_D = mg/L DO of dilution water = 9.0

S = mg/L DO of sample = assumed in this case to be zero

Therefore:

$$\frac{7.50 - 2.25}{9.0 - 2.0} \times 300 - 9 + 0 = \text{mg/L BOD} = 216 \text{ mg/L BOD}$$

Using the equation below:

(slope x 300) – Y-Intercept + sample DO = mg/L BOD

To determine slope, arbitrarily select point A in Figure 1. At this point the mg/L DO remaining is equal to 3.0 mg/L. The mL of sample at this point is 8 mL.The difference between the y-intercept of 9.0 mg/L and 3.0 mg/L equals 6 mg/L; 6 mg/L divided by 8 mL = 0.75 mg/L per mL.

slope = 0.75 mg/L per mL

Y intercept = 9.0 mg/L

sample DO = 0 (Because the sample is domestic sewage, this is assumed to be zero.)

Therefore:

(0.75 x 300) – 9.0 + 0 = mg/L BOD = 216 mg/L BOD

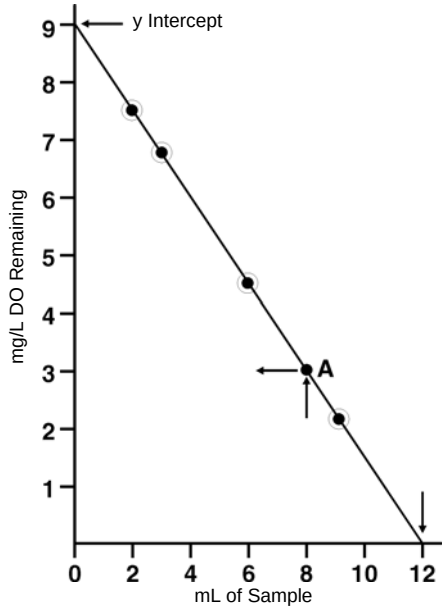


Figure 16 Dissolved Oxygen per mL of Sample

Summary of method

Biochemical Oxygen Demand (BOD) is a measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters. The BOD test is of limited value in measuring the actual oxygen demand because temperature change, biological population, water movement, sunlight, oxygen concentration, and other environmental factors cannot be reproduced accurately in the laboratory. The BOD test is of greatest value after patterns of oxygen uptake for a specific effluent and receiving water have been established.

The BOD test is performed by incubating a sealed wastewater sample (or a prepared dilution) for the standard five-day period and then determining the change in dissolved oxygen content. The BOD value is then calculated from the results of the dissolved oxygen tests.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
BOD Nutrient Buffer Pillows, for 3 liters of dilution water	1 pillow	50/pkg	1486166

Required apparatus

Description	Quantity/Test	Unit	Catalog number
BOD Bottle, glass-stoppered, 300-mL, unlabelled	6	6/pkg	62106
BOD Bottle Cap	6	6/pkg	241906
Bottle, wash, 500-mL	1	each	62011
Clippers, large	1	each	96800
Pipet, serological:			
Pipet, serological, 1-mL	1	each	919002
Pipet, serological, 5-mL	1	each	53237
Pipet, serological, 10-mL	1	each	53238
Pipet Filler	1	each	1218900
Dissolved Oxygen measurement apparatus	—	—	—

Recommended standards

Description	Unit	Catalog number
BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL	16/pkg	1486510
ezGGA BOD Standard Ampules, 450 mg/L, 2 mL	20/pkg	—

Optional reagents and apparatus

Description	Unit	Catalog number
BOD Nutrient Buffer Pillows		
for 300 mL of dilution water	50/pkg	1486166
for 4 liters of dilution water	50/pkg	2436466
for 6 liters of dilution water	50/pkg	1486266
for 19 liters of dilution water	25/pkg	1486398
Buffer Solution, APHA, for BOD, pH 7.2, phosphate type	500 mL	43149
Calcium Chloride Solution, APHA, for BOD	500 mL	42849
Ferric Chloride Solution, APHA, for BOD	1 L	42953
Magnesium Sulfate Solution, APHA, for BOD	500 L	43049
Nitrification Inhibitor	35 g	253335
Dispenser Cap, for Nitrification Inhibitor	each	45901
Potassium Iodide Solution, 100-g/L	500 mL	1228949
Sodium Hydroxide, pellets, ACS	500 g	18734
Sodium Hydroxide Standard Solution, 1.000 N	100 mL MDB	104532
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	35253
Starch Indicator Solution	100 mL MDB	34932
Sulfuric Acid Standard Solution, 0.020 N	1 L	20353
Sulfuric Acid Standard Solution, 1.000 N	1 L	127053
Potassium Permanganate	454g	16801H
Bottle, BOD, Serialized: 1-24[1]	24/pkg	1486610
Bottle, BOD, Disposable	117/case	2943100
Stopper for Disposable BOD Bottle	25/pkg	2943900
Bottle Rack, BOD, 12 bottle	each	2094200
Brush, cylinder, 2-in. diameter	each	68700
Incubator, BOD, Compact Model 205, 110 Vac	each	2616200
Incubator, BOD, Compact Model 205, 220/240 Vac	each	2616202
Leash, rubber, for stopper & bottle	6/pkg	2091606
ATU (1-1-allyl-2-thiourea)	50 g	2845425
Nitrification Inhibitor	500 g	253334
BOD Seed Inoculum, Polyseed	50 capsules	2918700

[1] Other numerical series are available

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Edition 7

Oxygen Demand, Chemical

DOC316.53.01101

Manganese III Reactor Digestion Method (with optional chloride removal)[1]

Method 10067

30 to 1000 mg/L COD Mn

Scope and Application: For water and wastewater

[1] U.S. Patent 5,556,787

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 272 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
To determine if the sample contains chloride, use Quantab® Titrator Strips for low range chloride.
If the sample COD is expected to exceed 1000 mg/L, dilute the sample as described in the *Multiplication factors* table.
Homogenize the sample for even distribution of solids and better accuracy and reliability.
Run one blank with each lot of reagent. Run all samples and blanks with the same lot of vials. The lot number appears on the container label.
The stability of the reagent blank allows for reuse. Verify the reagent blank quality by measuring the absorbance of the blank vs. a clean COD vial filled with deionized water. The absorbance range should be about 1.41–1.47.
If the sample boils during the digestion, the vial is not properly sealed. Test results will be invalid.
Spilled reagent will affect test accuracy and is hazardous. Do not run tests with spilled vials.
The maximum range of the VPD gauge is 40 inches of water; it will not indicate the full vacuum level obtained. Full vacuum is 20–25 inches of mercury; this can be measured at the vacuum pump with a gauge calibrated for inches of mercury.

Collect the following items:

Description	Quantity
Blender	1
DRB200 Reactor	1
Forceps, extra fine	1
Light Shield or Adapter (see *Instrument-specific information*)	1
Manganese III COD Reagent Vials, 20–1000 mg/L COD	1
Pipet, TenSette® (0.1 to 1.0 mL and 1.0 to 10.0 mL)	1 each
Pipet Tips for TenSette Pipet	2 of each
Sulfuric Acid, concentrated, ACS	1 mL
Test Tube Rack	1
Vacuum pretreatment device	1
Vacuum Pump	1
Vial, glass, for sample and acid	2
Water, deionized	varies

See *Consumables and replacement items* for reorder information.

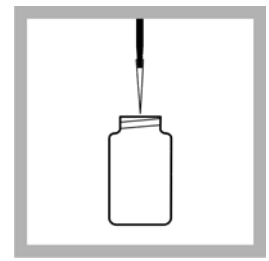
Acidified sample preparation

13. Turn on the DRB200 Reactor and heat to 150 °C or set to COD program.

14. Homogenize 100 mL of sample for 30 seconds in a blender.

If suspended solids are present, continue to mix the sample while pipetting.

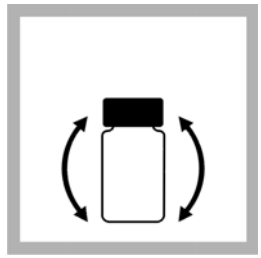
15. Blank Preparation: Pipet 9.0 mL of deionized water into an empty glass mixing cell.



16. Prepared Sample: Pipet 9.0 mL of homogenized sample into another empty glass mixing cell

Acidified sample preparation (continued)

17. Using an automatic dispenser or TenSette® Pipet, add 1.0 mL of concentrated sulfuric acid to both the sample and the blank.



18. Cap the cells tightly and invert several times.

The solution will become hot. Cool to room temperature before proceeding.

Acidified samples are stable for several months when refrigerated at 4 °C.

Proceed to *Vacuum pretreatment*.

Calculate the multiplication factor

All dilutions require that the ratio of sample to sulfuric acid remain at 9:1. For other dilutions that are not listed in the *Multiplication factors* table, add the sample volume and the deionized water and divide by the sample volume to obtain the multiplication factor.

Note: *Mixing concentrated sulfuric acid and water is not additive. Adding 1.0 mL of concentrated sulfuric acid to 9.0 mL of sample does not result in a final volume of 10.0 mL. This factor is built into the calibration curve.*

Table 273 Multiplication factors

Sample (mL)	Deionized Water (mL)	Range (mg/L COD)	Multiplication Factor
6.0	3.0	30–1500	1.5
3.0	6.0	60–3000	3
1.0	8.0	180–9000	9
0.5	8.5	360–18,000	18

For best results, use 0.5 mL or more of sample for diluting. If sample values exceed 18,000 mg/L COD, use a separate sample dilution before performing the sample chloride removal procedure.

Example: Dilute the sample to a range of 90–4500 mg/L COD.

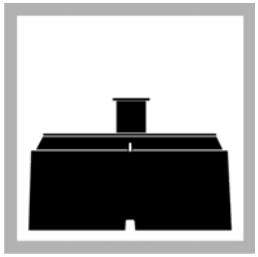
Sample Volume (2.0 mL) + Deionized water (7.0 mL) = Total Volume (9.0 mL)

$$\text{Multiplication Factor} = \frac{\text{Total Volume}}{\text{Sample Volume}} = \frac{9.0\ \text{mL}}{2.0\ \text{mL}} = 4.5$$

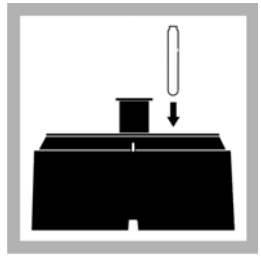
Standard test range is 50 to 1000 mg/L COD.

Example test range = 4.5(50) to 4.5(1000) = 225 to 4500 mg/L COD

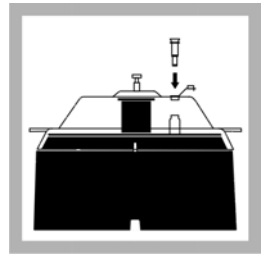
Vacuum pretreatment



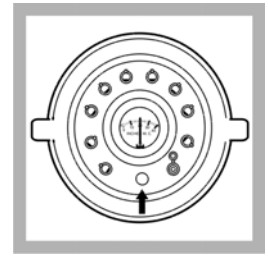
1. Attach the Vacuum Pretreatment Device (VPD) to a vacuum pump (not an aspirator-type vacuum) that can create a vacuum of 20–25 inches of mercury.



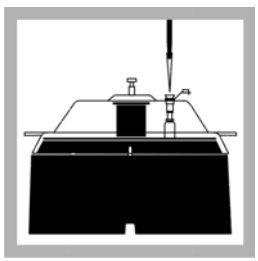
2. Label each Mn III COD vial and remove the cap. Insert the vials in the numbered holes in the VPD base.



3. Place the VPD top on the base. Insert a fresh Chloride Removal Cartridge (CRC) directly above each Mn III COD Reagent Vial. Plug any open holes in the VPD top using the stoppers provided.

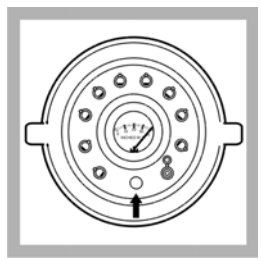


4. Turn on the vacuum pump and adjust the vacuum regulator valve on top of the VPD until the internal gauge reads 20 inches of water.

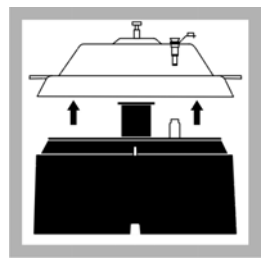


5. Pipet 0.60 mL of acidified sample (see *Acidified sample preparation*) into the CRC. Pipet 0.60 mL of acidified blank into another CRC. It should take 30–45 seconds to draw the liquid through the CRC into each vial.

If the sample does not flow through the Chloride Removal Cartridge (CRC), increase the vacuum until flow starts, then reduce the vacuum down to 20 inches of water. Proceed as usual.



6. Close the vacuum regulator valve completely to achieve full vacuum. After one minute of full vacuum, slide the VPD back and forth several times to dislodge any drops clinging to the cartridge.



7. Open the VPD regulator valve to release the vacuum. Turn the pump off. Remove the VPD top and set it beside the base.

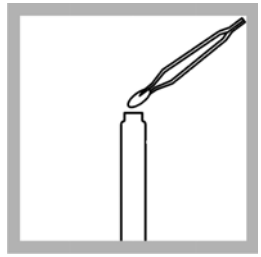
Dispose of the used Chloride Removal Cartridge. Do not reuse it.

Proceed to *Sample preparation and measurement*.

Sample preparation and measurement

1. Use forceps to remove the filter from the top of each CRC.

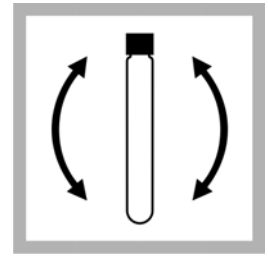
If the sample does not contain suspended solids, it is not necessary to transfer the filter to the digestion vial.



2. Insert each filter in the corresponding Mn III COD Vial. (Use numbers on the VPD as a guide.)

To avoid cross-contamination between samples, clean forcep tips between samples by wiping with a clean towel or rinsing with deionized water.

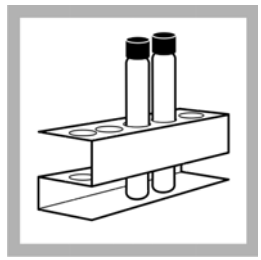
3. Remove the Mn III COD vial from the vacuum chamber and replace the original cap. Screw the cap on tightly.



4. Invert the vials several times to mix.

5. Insert the vials in the DRB200 Reactor at 150 °C. Close the protective cover. Digest for one hour.

To oxidize resistant organics, samples can be digested for up to four hours. Digest the blank for the same time period as the samples.



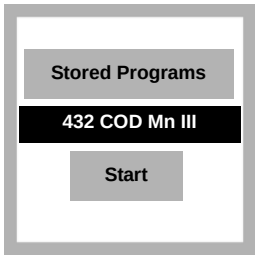
6. Insert the vials in a cooling rack for two minutes.

If the solution develops a colorless upper layer and a purple lower layer, invert the vial several times to mix and proceed to the next step.

7. Cool the vials to room temperature in a cool water bath or with running tap water for several minutes.

8. Remove the vials from the water and wipe with a clean, dry paper towel.

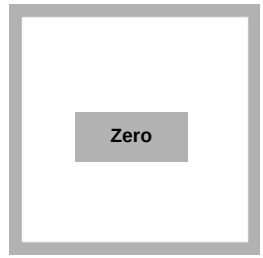
Sample preparation and measurement (continued)



9. Select the test.

Insert an adapter or a light shield if required (see *Instrument-specific information*).

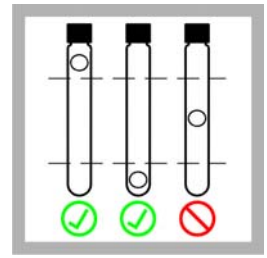
10. Insert the blank into the 16-mm cell holder.



11. **ZERO** the instrument.

The display will show:

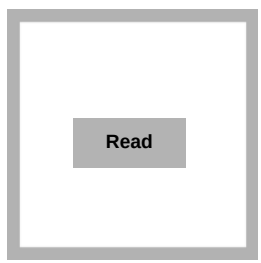
0 mg/L COD Mn



12. Make sure the filter disc is not suspended in the middle of the vial; it can interfere with the instrument reading.

The disc must be more than 20 mm (0.8") or less than 10 mm (0.4"), from the bottom of the vial. Move it by gently swirling or by lightly tapping the vial on the table top.

13. Wipe the prepared sample and insert it into the .



14. **READ** the results in mg/L COD Mn.

Interferences

Inorganic materials may also be oxidized by trivalent manganese and constitute a positive interference when present in significant amounts. Chloride is the most common interference and is removed by sample pretreatment with the Chloride Removal Cartridge. If chloride is known to be absent or present in insignificant levels, the pretreatment can be omitted. A simple way to determine if chloride will affect test results is to run routine samples with and without the chloride removal, then compare results. Other inorganic interferences (i.e., nitrite, ferrous iron, sulfide) are not usually present in significant amounts. If necessary, these interferences can be corrected after determining their concentrations with separate methods and adjusting the final COD test results accordingly.

Ammonia nitrogen is known to interfere in the presence of chloride; it does not interfere if chloride is absent.

Sample collection, preservation and storage

- Collect samples in clean glass bottles.
- Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples containing solids to assure representative samples.
- Samples treated with concentrated sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C may be stored up to 28 days.
- Correct test results for volume additions.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- COD standard solution, 800 mg/L

3. Use 0.60 mL of the 800-mg/L solution in place of the sample.
4. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
432	DR 5000	600 mg/L COD	576–624 mg/L COD	8 mg/L COD

Estimated detection limit (EDL)

The EDL for program 432 is 4 mg/L COD. The EDL is the calculated lowest average concentration in a deionized water matrix that is different from zero with a 99% level of confidence.

Summary of method

Chemical Oxygen Demand (COD) is defined as "... a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant" (APHA Standard Methods, 19th ed., 1995). Trivalent manganese is a strong, non-carcinogenic chemical oxidant that changes quantitatively from purple to colorless when it reacts with organic matter. It typically oxidizes about 80% of the organic compounds. Studies have shown that the reactions are highly reproducible and test results correlate closely to Biochemical Oxygen Demand (BOD) values and hexavalent chromium COD tests. None of the oxygen demand tests provide 100% oxidation of all organic compounds.

A calibration is provided which is based on the oxidation of Potassium Acid Phthalate (KHP). A different response may be seen in analyzing various wastewaters. The KHP calibration is adequate for most applications. The highest degree of accuracy is obtained when test results are correlated to a standard reference method such as BOD or one of the chromium COD methods. Special waste streams or classes will require a separate calibration to obtain a direct mg/L COD reading or to generate a correction factor for the precalibrated KHP response. The

sample digestion time can be extended up to four hours for samples that are difficult to oxidize. Test results are measured at 510 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Manganese III COD Reagent Vials, 20–1000 mg/L COD	1	25/pkg	2623425
Chloride Removal Cartridge (CRC)	1	25/pkg	2661825
Sulfuric Acid, concentrated, ACS	1 mL	2.5 L	97909
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Blender, 120 VAC	1	each	2616100
Cap, with inert Teflon liner, for mixing bottle	varies	12/pkg	2401812
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Forceps, extra fine point	1	each	2669600
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips for TenSette Pipet 19700-10	2	50/pkg	2199796
Pipet, TenSette, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips for TenSette Pipet 19700-01	2	50/pkg	2185696
Test Tube Rack	1	each	1864100
Vacuum Pretreatment Device (VPD)	1	each	4900000
Vacuum Pump, 1.2 CFM 115V	1	each	2824800
Vial, glass, for sample plus acid	2	each	2427700

Recommended standards

Description	Unit	Catalog number
COD Standard Solution, 800-mg/L COD	200 mL	2672629
Oxygen Demand Standard for BOD, COD, TOC, 10-mL ampules	16/pkg	2833510
Potassium Acid Phthalate, ACS	500 g	31534
Wastewater Standard, Influent Inorganics, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149

Optional reagents and apparatus

Description	Unit	Catalog number
Dispenser, automatic, 1.0–5.0 mL	each	2563137
Titrator Strips, Quantab®, for low range chloride	40 tests	2744940
Finger cots	2/pkg	1464702
Flask, volumetric class A, 1 L	each	1457453

Optional reagents and apparatus

Description	Unit	Catalog number
pH Paper, 0–14 pH range	100/pkg	2601300
Balance, analytical, 80 g capacity, 115 VAC	each	2936701
Weighing Paper, 76x76 mm	500/pkg	1473800
Oven, Laboratory 120 VAC/60Hz	each	1428900
Oven, Laboratory 240 VAC/50Hz	each	1428902
COD Standard Solution, 300-mg/L	200 mL	1218629
COD Standard Solution, 300-mg/L	500 mL	1218649
COD Standard Solution, 1000-mg/L	200 mL	2253929
Standard Methods book, most recent edition	each	2270800
Pipet Tips for TenSette Pipet 19700-10	250/pkg	2199725
Pipet Tips for TenSette Pipet 19700-01	1000/pkg	2185628

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Edition 7

Oxygen Demand, Chemical

(Manganese III Reactor Digestion Method)

DOC316.53.01102

Manganese III Reactor Digestion Method (without chloride removal)[1]

Method 10067

30 to 1000 mg/L COD Mn

Scope and Application: For water and wastewater

[1] U.S. Patent 5,556,787

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 274 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

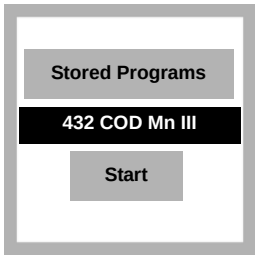
DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
If the sample contains chloride, use the chloride removal method. To determine if the sample contains chloride, use Quantab® Titrator Strips for low range chloride.
If the sample COD value is not between 30 and 1000 mg/L, dilute the sample with deionized water to obtain this range. Multiply the final result by the dilution factor.
Homogenize the sample for even distribution of solids and better accuracy and reliability.
Stability of the reagent blank allows for reuse. Verify the reagent blank quality by measuring the absorbance of the blank vs. a clean COD vial filled with deionized water. The absorbance range should be about 1.4–1.5.
If the sample boils during the digestion, the vial is not properly sealed. Test results will be invalid.
Use finger cots to handle hot sample cells.
Spilled reagent will affect test accuracy and is hazardous. Do not run tests with spilled vials.
See the DRB200 User Manual for selecting pre-programmed temperature applications.

Collect the following items:

Description	Quantity
Blender	1
DRB200 Reactor	1
Light Shield	1
Manganese III COD Reagent Vials, 20–1000 mg/L COD	1
Pipet, TenSette® (0.1 to 1.0 mL)	1
Pipet tips for TenSette Pipet	2
Test Tube Rack	1
Water, deionized	varies

See *Consumables and replacement items* for reorder information.

Sample preparation and measurement



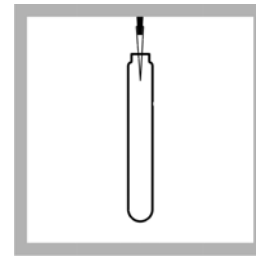
15. Select the test.

Insert an adapter or a light shield if required (see *Instrument-specific information*).

16. Turn on the DRB200 Reactor and heat to 150 °C or use the COD program.

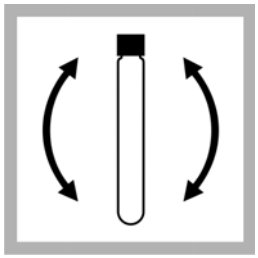
17. Homogenize 100 mL of sample for 30 seconds in a blender.

If suspended solids are present, continue to mix the sample while pipetting.



Pipet 0.5 mL of homogenized sample into one Mn III COD vial (the prepared sample) and 0.5 mL of deionized water into another Mn III COD vial (the blank).

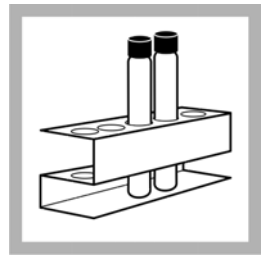
Sample preparation and measurement (continued)



18. Cap and invert several times to mix.

19. Insert the vials in the DRB200 Reactor at 150 °C. Close the protective cover. Digest for one hour.

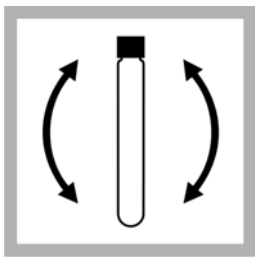
Digest more resistant organics and the blank for up to four hours.



20. Remove the vials and place them in a cooling rack for two minutes.

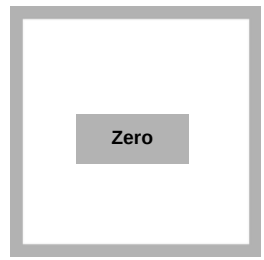
If a vial develops a colorless upper layer and a purple lower layer, invert the vial several times to mix and proceed.

21. Cool the vials to room temperature in a cool water bath or with running tap water. This takes several minutes.



22. Invert the vials several times to mix.

23. Wipe the blank and insert it into the 16-mm round cell holder.



24. **ZERO** the instrument.

The display will show:

0 mg/L COD Mn

25. Wipe the sample and insert it into the 16-mm round cell holder.

READ the results in mg/L COD Mn.

Interferences

Inorganic materials may also be oxidized by trivalent manganese and constitute a positive interference when present in significant amounts. Chloride is the most common interference. If chloride is known to be present in significant levels, the chloride needs to be removed with the vacuum pretreatment device. A simple way to determine if chloride will affect test results is to run routine samples with and without the chloride removal, then compare results. Other inorganic interferences (i.e., nitrite, ferrous iron, sulfide) are not usually present in significant amounts. If necessary, these interferences can be corrected after determining their concentrations with separate methods and adjusting the final COD test results accordingly.

Ammonia nitrogen is known to interfere in the presence of chloride; it does not interfere if chloride is absent.

Sample collection, preservation and storage

- Collect samples in clean glass bottles.
- Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples containing solids to assure representative samples.
- Samples treated with concentrated sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C may be stored up to 28 days.
- Correct test results for volume additions.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- COD Standard Solution, 800 mg/L

6. Use 0.50 mL of the 800-mg/L solution in place of the sample.
7. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
8. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—∆Concentration per 0.010 ∆Abs
432	600 mg/L COD	576–624 mg/L COD	8 mg/L COD

Estimated detection limit (EDL)

The EDL for program 432 is 4mg/L COD. The EDL is the calculated lowest average concentration in a deionized water matrix that is different from zero with a 99% level of confidence.

Summary of method

Chemical Oxygen Demand (COD) is defined as "... a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant" (APHA Standard Methods, 19th ed., 1995). Trivalent manganese is a strong, non-carcinogenic chemical oxidant that changes quantitatively from purple to colorless when it reacts with organic matter. It typically oxidizes about 80% of the organic compounds. Studies have shown that the reactions are highly reproducible and test results correlate closely to Biochemical Oxygen Demand (BOD) values and hexavalent chromium COD tests. None of the oxygen demand tests provide 100% oxidation of all organic compounds.

A calibration is provided which is based on the oxidation of Potassium Acid Phthalate (KHP). A different response may be seen in analyzing various wastewaters. The KHP calibration is adequate for most applications. The highest degree of accuracy is obtained when test results are correlated to a standard reference method such as BOD or one of the chromium COD methods. Special waste streams or classes will require a separate calibration to obtain a direct mg/L COD reading or to generate a correction factor for the precalibrated KHP response. The sample digestion time can be extended up to four hours for samples that are difficult to oxidize. Test results are measured at 510 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Manganese III COD Reagent Vials, 20–1000 mg/L COD	1	25/pkg	2623425
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Blender, 2-speed, 120 VAC	1	each	2616100
Blender, 2-speed, 240 VAC	1	each	2616102
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 19700-01	2	50/pkg	2185696
Test Tube Rack	1	each	1864100

Recommended standards and apparatus

Description	Unit	Catalog number
COD Standard Solution, 800-mg/L COD	200 mL	2672629
Oxygen Demand Standard for BOD, COD, TOC, 10-mL ampules	16/pkg	2833510
Potassium Acid Phthalate, ACS	500 g	31534
Wastewater Standard, Influent Inorganics, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149
Goggles, Safety vented	each	2550700
Gloves, Chemical Resistant, size 9-9½[1]	1 pair	2410104

[1] other sizes are available

Optional reagents and apparatus

Description	Unit	Catalog number
Titrator Strips, Quantab®, for low range chloride	40 tests	2744940
Finger cots	2/pkg	1464702
Flask, volumetric class A, 1L	each	1457453
pH Paper, 0–14 pH range	100/pkg	2601300
Pipet Tips for TenSette Pipet 1970001	1000/pkg	2185628
Balance, analytical, 80 g capacity, 115 VAC	each	2936701
Weighing Paper, 76x76 mm	500/pkg	1473800
Oven, Laboratory 120 VAC/60Hz	each	1428900
Oven, Laboratory 240 VAC/50Hz	each	1428902
COD Standard Solution, 300-mg/L	200 mL	1218629
COD Standard Solution, 300-mg/L	500 mL	1218649
COD Standard Solution, 1000-mg/L	200 mL	2253929
Standard Methods book, most recent edition	each	2270800

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Edition 7

Oxygen Demand, Chemical

DOC316.53.01099

USEPA[1] Reactor Digestion Method[2] **Method 8000**

**0.7 to 40.0[3] mg/L COD (ULR); 3 to 150 mg/L COD (LR);
20 to 1500 mg/L COD (HR); 200 to 15,000 mg/L COD (HR Plus)**

Scope and Application: For water, wastewater; digestion is required

[1] Ranges 3 to 150 mg/L COD and 20 to 1500 mg/L COD are USEPA approved for wastewater analyses (Standard Method 5220 D), *Federal Register*, April 21, 1980, 45(78), 26811-26812.

[2] Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397

[3] The ULR range is not available on the DR 2700 or the DR/2400.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test

Table 275 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and associated MSDS sheets.
Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number appears on the container label. See *Blanks for colorimetric determination*.
Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Be prepared to wash spills with running water.
Wear appropriate eye protection and clothing for adequate user protection. If contact occurs, flush the affected area with running water. Review and follow reagent MSDS safety instructions carefully.
Store unused (light sensitive) vials in a closed box.
If high chloride samples are being tested, refer to the Alternate reagents section.

Collect the following items:

Description	Quantity
Beaker, 250-mL	1
Blender	1
COD Digestion Reagent vials	varies
DRB200 Reactor	1
Light Shield or adapter (see *Instrument-specific information*)	1
Magnetic stirrer and stir bar	1
Opaque shipping container for storage of unused, light-sensitive reagent vials	varies
Pipet, TenSette®, 0.1 to 1.0 mL, with tips (for 200–15,000 mg/L range)	1
Pipet, volumetric, 2.00 mL	2
Pipet Filler, safety bulb	1
Test Tube Rack	2

See *Consumables and replacement items* for reorder information.

Reactor digestion procedure

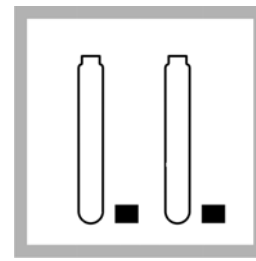
1. Homogenize 100 mL of sample for 30 seconds in a blender. For samples containing large amounts of solids, increase the homogenization time.

If the sample does not contain suspended solids, omit steps 1 and 2.

2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.

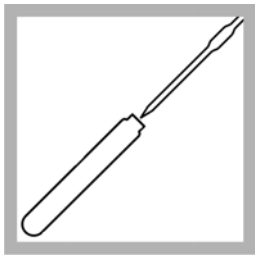
3. Turn on the DRB200 Reactor. Preheat to 150 °C.

See the DRB200 User Manual for selecting pre-programmed temperature applications.



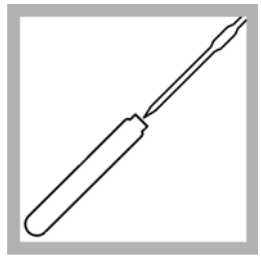
4. Remove the caps from two COD Digestion Reagent Vials. (Be sure to use vials for the appropriate range.)

Reactor digestion procedure (continued)



5. Prepared Sample: Hold one vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of sample to the vial.

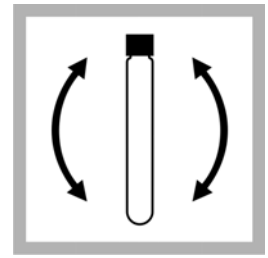
For the 200–15,000 mg/L vials: Use a TenSette® Pipet to add 0.20 mL of sample to the vial.



6. Blank Preparation: Hold a second vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of deionized water to the vial.

For the 200–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of deionized water to the vial.

7. Cap the vials tightly. Rinse them with water and wipe with a clean paper towel.



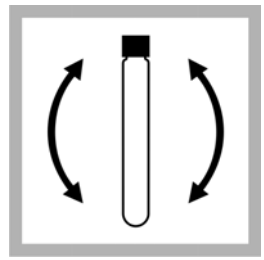
8. Hold the vials by the cap over a sink. Invert gently several times to mix. The sample vials become very hot during mixing.

Insert the vials in the preheated DRB200 Reactor. Close the protective lid.

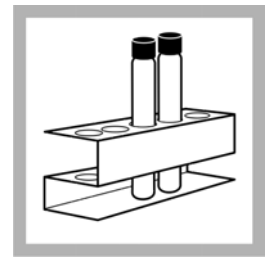
9. Heat the vials for two hours.

10. Turn the reactor off.

Before removing the vials, wait about 20 minutes for the vials to cool to 120 °C or less.



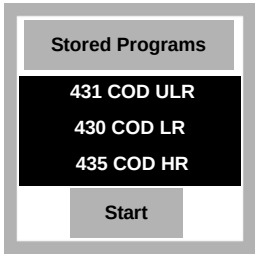
11. Invert each vial several times while still warm.



12. Place the vials into a rack and cool to room temperature.

Proceed to *Colorimetric determination*.

Colorimetric determination



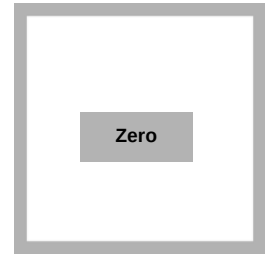
1. Select the test.

Insert an adapter or light shield if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Clean the outside of the vials with a damp towel followed by a dry one.

3. Insert the blank into the 16-mm cell holder.



4. **ZERO** the instrument.

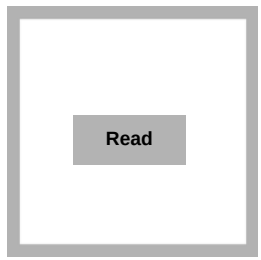
The display will show:

0 mg/L COD

or

0.0 mg/L COD

5. Insert the sample vial into the 16-mm .



6. **READ** the results in mg/L COD.

7. If using High Range Plus COD Digestion Reagent Vials, multiply the result by 10.

For most accurate results with samples near 1500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.

Blanks for colorimetric determination

The blank may be used repeatedly for measurements using the same lot of vials. Store the blank in the dark.

1. Monitor decomposition by measuring the absorbance at the appropriate wavelength. Refer to the *Range-specific test wavelengths* table.
2. Zero the instrument in the absorbance mode. Use a vial containing 5 mL of deionized water and measure the absorbance of the blank. Record the value.
3. Prepare a new blank when the absorbance has changed by about 0.01 absorbance units.

Interferences

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in *Column 1* of the *Interfering substances* table. Dilute samples with higher chloride concentrations. Dilute the sample enough to reduce the chloride concentration to the level given in *Column 2*.

Note: *For best results, use the low range and ultra-low range test for samples with high chloride concentrations (approaching maximum concentration) and low COD concentrations.*

If sample dilution will cause the COD concentration to be too low for accurate determination, add 0.50 g of mercuric sulfate ($HgSO_4$) to each COD vial before the sample is added.

The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in *Column 3*.

Table 276 Interfering substances

Vial range	**Column 1** (Maximum chloride concentration)	**Column 2** (Suggested chloride concentration for diluted samples)	**Column 3** (Maximum chloride concentration with mercuric sulfate)
Ultra Low Range [1] (0.7–40.0 mg/L)	2000	1000	N/A
Low Range (3–150 mg/L)	2000	1000	8000
High Range (20–1500 mg/L)	2000	1000	4000
High Range Plus (200–15,000 mg/L)	20,000	10,000	40,000

[1] Ultra Low Range is not available on the DR 2700.

Sample collection, preservation and storage

- Collect samples in glass bottles.
- Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples containing solids to assure representative samples.
- Samples treated with sulfuric acid* to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days.
- Correct the test results for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 1000 mg/L COD Standard Solution

  OR

- Potassium acid phthalate (KHP), dried overnight at 120 °C
- Deionized water, organic free
- Class A volumetric flasks
- Class A volumetric pipet

0.7 to 40.0 mg/L range

1. Prepare a 30-mg/L COD standard solution as follows:
   e. Pipet 3.00 mL of the 1000 mg/L standard into a 100-mL volumetric flask.
   f. Dilute to volume with deionized water and mix well.
2. Use 2 mL of the 30 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 30 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

3 to 150 mg/L range

1. Prepare a 100-mg/L COD standard solution as follows:
   a. Pipet 10 mL of the 1000 mg/L standard into a 100-mL volumetric flask.
   b. Dilute to volume with deionized water and mix well.
2. Use 2 mL of the 100-mg/L solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 100 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard

20 to 1500 mg/L range

Use 2 mL of 300 mg/L, 800 or 1000 mg/L COD standards for accuracy check.

200 to 15,000 mg/L range

1. Prepare a 10,000-mg/L COD standard solution as follows:

   a. Dissolve 8.500 g of dried KHP in 1000-mL of organic-free deionized water.

2. Use 0.2 mL of the 10,000 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 10,000 mg/L (after multiplying by 10). Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

Standard adjust

1. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.

2. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Alternate reagents

Mercury-free COD2 Reagents can provide a mercury-free testing option for non-reporting purposes. For process control applications, COD2 Reagents will eliminate mercury waste and save on disposal costs. These reagents are fully compatible with test procedures and calibration curves programmed into the spectrophotometer. Determine chloride and ammonia for accurate results.

Important Note: *COD2 reagents are not approved for USEPA reporting purposes. Because COD2 reagents do not contain mercury as a masking agent, they exhibit a positive interference from chloride. Request a copy of the COD2 Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.*

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
430 (Range, 3–150 mg/L)	80 mg/L COD (Low Range)	77–83 mg/L COD	3 mg/L COD
431 (Range, 0.5–40.0 mg/L)	30 mg/L COD (Ultra Low Range)	28.8–31.2 mg/L COD	0.5 mg/L COD
435 (Range, 20–1500 mg/L)	800 mg/L COD (High Range)	785–815 mg/L COD	23 mg/L COD
435 (Range, 200–15,000 mg/L)	8000 mg/L COD (High Range Plus)	7850–8150 mg/L COD	230 mg/L COD

Summary of method

The results in mg/L COD are defined as the milligrams of O_2 consumed per liter of sample under the conditions of this procedure. The sample is heated for two hours with sulfuric acid and a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}).

When the 0.7–40.0 or the 3–150 mg/L colorimetric method is used, the amount of Cr^{6+} remaining is determined. When the 20–1500 mg/L or 200–15,000 mg/L colorimetric method is used, the amount of Cr^{3+} produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

Test results are measured at the wavelengths specified in the *Range-specific test wavelengths* table.

Table 277 Range-specific test wavelengths

Range in mg/L COD	Wavelength
0.7 to 40.0 mg/L[1]	350 nm
3 to 150 mg/L	420 nm
20 to 1500	620 nm
2000 to 15,000 mg/L	620 nm

[1] Not available on the DR 2700

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Select the appropriate COD Digestion Reagent Vial:			
Ultra Low Range, 0.7 to 40 mg/L COD	1–2 vials	25/pkg	2415825
Low Range, 3 to 150 mg/L COD	1–2 vials	25/pkg	2125825
High Range, 20 to 1500 mg/L COD	1–2 vials	25/pkg	2125925
High Range Plus, 200 to 15,000 mg/L COD	1–2 vials	25/pkg	2415925
Water, deionized	varies	4 L	27256

Alternate reagents[1]

Description	Quantity/Test	Unit	Catalog number
Select the appropriate COD Digestion Reagent Vial:			
COD2, Low Range, 0 to 150 mg/L COD	1–2 vials	25/pkg	2565025
COD2, High Range, 0 to 1500 mg/L COD	1–2 vials	25/pkg	2565125
COD2, High Range, 0 to 1500 mg/L COD	1–2 vials	150/pkg	2565115
COD2, High Range Plus, 0 to 15,000 mg/L COD	1–2 vials	25/pkg	2834325
COD Digestion Reagent Vials, 3 to 150 mg/L COD	1–2 vials	150/pkg	2125815
COD Digestion Reagent Vials, 200 to 1500 mg/L COD	1–2 vials	150/pkg	2125915
COD Digestion Reagent Vials, ULR 0.7-40.0 mg/L	1-2 vials	150/pkg	2415815
COD Digestion Reagent Vials, HR plus,200-25,000 mg/L	1-2 vials	150/pkg	2415915

[1] These reagents are not approved for USEPA reporting purposes. Request a copy of the COD2 Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.

Required apparatus

Description	Quantity	Unit	Catalog number
Blender, 2-speed, 120 VAC	1	each	2616100
OR			
Blender, 2-speed, 240 VAC	1	each	2616102
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Pipet Filler, safety bulb	1	each	1465100
Pipet, Volumetric, Class A, 2.00 mL	1	each	1451536

Recommended standards and apparatus

Description	Unit	Catalog number
Beaker, 250-mL	each	50046H
COD Standard Solution, 300-mg/L	200 mL	1218629
COD Standard Solution, 300-mg/L	500mL	1218649
COD Standard Solution, 800-mg/L	200 mL	2672629
COD Standard Solution, 1000-mg/L	200 mL	2253929
Oxygen Demand Standard (BOD, COD, TOC), 10-mL ampules	16/pkg	2833510
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Potassium Acid Phthalate, ACS	500 g	31534
Stir Bar, 28.6 x 7.9 mm	each	2095352
Stirrer, Electromagnetic, 120 VAC, with electrode stand	each	4530001
Stirrer, Electromagnetic, 230 VAC, with electrode stand	each	4530002
Test Tube Rack, 16 mm	each	1864100
Wipes, Disposable	70/pkg	2096900

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity, 115 VAC	each	2936701
Flask, volumetric, 1000 mL Class A	each	1457453
Flask, volumetric, 100-mL Class A	each	1457442
Mercuric Sulfate	28 g	191520
Pipet, volumetric, 3-mL, Class A	each	1451503
Pipet, volumetric, 10-mL, Class A	each	1451538
Sulfuric Acid, conc	500 mL	97949
Wastewater Influent Standard for mixed parameters NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500mL	2833149
Wastewater Effluent Standard, for mixed parameters NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500mL	2833249
Weighing Paper, 76x76 mm	500/pkg	1473800
Finger cots	2/pkg	1464702
Gloves, chemical resistant 9-9 ½"[1]	1 pair	2410104
Safety goggles, vented	each	2550700
EZ COD™ Recycling Service with 5-gal[2] bucket–mail back[3]	each	2895405
EZ COD™ Recycling Service with 5-gal bucket–pick up[3]	each	2895405P

[1] Other sizes are available

[2] 20 and 55 gal are available.

[3] ez COD available to U.S. customers only

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Edition 7

Oxygen, Dissolved

DOC316.53.01096

HRDO Method **Method 8166**

HR (0.3 to 15.0 mg/L O_2) **AccuVac® Ampuls**

Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 278 Instrument-specific information

Instrument	AccuVac Ampuls	
	Sample cell	Adapter
DR 6000	2427606	—
DR 5000	2427606	—
DR 3900	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2122800	LZV584 (C)

Before starting the test:

Analyze samples on-site. Do not store for later analysis

Collect the following items:

Description	Quantity
High Range Dissolved Oxygen AccuVac® Ampuls with reusable Ampul caps	1
Polypropylene Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1

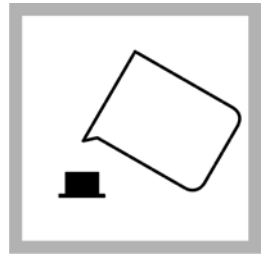
See *Consumables and replacement items* for reorder information.

HRDO Method

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. Blank Preparation: Fill a sample cell with 10 mL of sample.

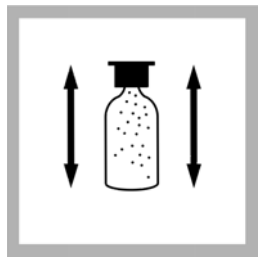


3. Fill a blue Ampul cap with sample.

4. Prepared Sample: Fill a High Range Dissolved Oxygen AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

5. Hold the Ampul with the tip pointing down and immediately insert the Ampul into the Ampul cap.

The cap prevents contamination from atmospheric oxygen.

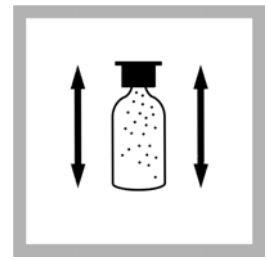


6. Shake the Ampul for 30 seconds.

A small amount of undissolved reagent will not affect results.

7. Start the instrument timer.

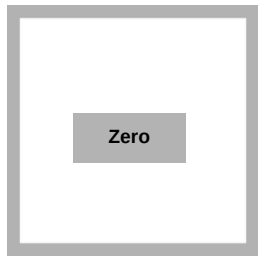
A two-minute reaction period will begin. This enables the oxygen that was degassed during aspiration to redissolve and react.



8. When the timer expires, shake the Ampul for 30 seconds.

Allow any bubbles to dissipate before proceeding.

9. Insert the blank in the cell holder.

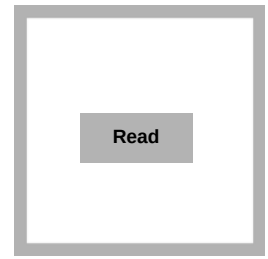


10. ZERO the instrument.

The display will show:

0.0 mg/L O_2

11. Insert the prepared sample into the cell holder.



12. READ the results in mg/L O_2.

Interferences

Table 279 Interfering substances

Interfering substance	Interference level
Cr^{3+}	Greater than 10 mg/L
Cu^{2+}	Greater than 10 mg/L
Fe^{2+}	Greater than 10 mg/L
Mg^{2+}	Magnesium is commonly present in seawater and causes a negative interference. If the sample contains more than 50% seawater, the oxygen concentration obtained by this method will be 25% less than the true oxygen concentration. If the sample contains less than 50% seawater, the interference will be less than 5%.
Mn^{2+}	Greater than 10 mg/L
Ni^{2+}	Greater than 10 mg/L
NO_2^-	Greater than 10 mg/L

Sample collection, preservation and storage

The main consideration in sampling with the High Range Dissolved Oxygen Ampul is to prevent the sample from becoming contaminated with atmospheric oxygen between breaking open the Ampul and reading the absorbance. This is accomplished by capping the Ampul with an Ampul cap. If the Ampul is securely capped, the Ampul should be safe from contamination for several hours. The absorbance will decrease by approximately 3% during the first hour and will not change significantly afterwards.

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested may change with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time and other factors. A single dissolved oxygen test rarely reflects the accurate overall condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results. Samples must be tested immediately upon collection, although only a small error results if the absorbance reading is taken several hours later.

Accuracy check

The results of this procedure may be compared with the results of a titrimetric procedure or by using a dissolved oxygen meter.*

Method performance

Program	Standard	95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
445	6.7 mg/L O_2	6.2–7.3 mg/L O_2	0.09 mg/L O_2

Summary of method

The High Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum-sealed in a 14-mL Ampul. When the AccuVac Ampul is opened in a sample containing dissolved oxygen, it forms a yellow color which turns purple. The purple color development is proportional to the concentration of dissolved oxygen. Test results are measured at 535 nm.

* See *Optional reagents, apparatus and meters*.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
High Range Dissolved Oxygen AccuVac® Ampuls	1	25/pkg	2515025

Required apparatus

Description	Quantity	Unit	Catalog number
Polypropylene Beaker, 50-mL	1	each	108041
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional reagents, apparatus and meters

Description	Unit	Catalog number
AccuVac® snapper	each	2405200
AccuVac sampler	each	2405100
AccuVac, vials for sample blanks	25/pkg	2677925
AccuVac stoppers	6/pkg	173106
HQ30d Meter with Standard LDO Dissolved Oxygen Probe[1] (1 meter cable)	each	HQ30d53301000
HQ40d Meter with Standard LDO Probe[1] (1 meter cable)	each	HQ40d53301000

[1] Additional probes and cable lengths are available.

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Edition 7

Oxygen, Dissolved

DOC316.53.01098

Indigo Carmine Method **Method 8316**

LR (6 to 800 µg/L O_2) **AccuVac® Ampuls**

Scope and Application: For boiler feedwater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 280 Instrument-specific information

Instrument	AccuVac Ampuls	
	Sample cell	Adapter
DR 6000	2427606	—
DR 5000	2427606	—
DR 3900	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2122800	LZV584 (C)

Before starting the test:

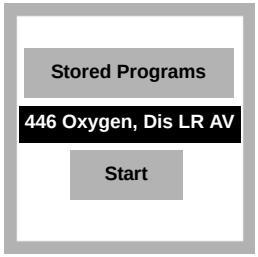
Analyze samples on-site. Do not store for later analysis

Collect the following items:

Description	Quantity
Low Range Dissolved Oxygen AccuVac® Ampuls	1
Polypropylene Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

Indigo Carmine method for AccuVac® Ampuls

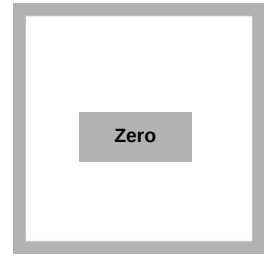


1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. Blank Preparation: Fill a sample cell with 10 mL of sample.

3. Insert the blank into the cell holder.



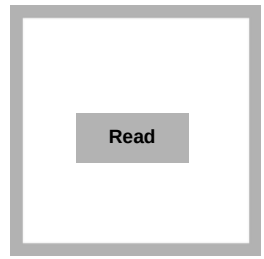
4. **ZERO** the instrument.

The display will show:

0 µg/L O_2

5. Fill a Low Range Dissolved Oxygen Ampul with sample. Keep the tip immersed while the Ampul fills completely.

6. Immediately insert the Ampul into the cell holder.



7. **READ** the results in µg/L O_2.

Use the initial reading. The reading is stable for 30 seconds. After 30 seconds the Ampul solution will absorb oxygen from the air.

Interferences

Excess amounts of thioglycolate, ascorbate, ascorbate + sulfite, ascorbate + cupric sulfate, nitrite, sulfite, thiosulfate and hydroquinone will not reduce the oxidized form of the indicator and do not cause significant interference.

Table 281 Interfering substances

Interfering substance	Interference level
Hydrazine	100,000 fold excess will begin to reduce the oxidized form of the indicator solution.
Sodium hydrosulfite	Reduces the oxidized form of the indicator solution and will cause a significant interference.

Sample collection, preservation and storage

The main consideration in this procedure is to prevent contaminating the sample with atmospheric oxygen.

- For best results, sample from a stream of water that is hard plumbed to the sample source.
- Use a funnel to maintain a continual flow of sample and yet collect enough sample to immerse the Ampul.
- Do not introduce air in place of the sample.
- Rubber tubing, if used, will introduce unacceptable amounts of oxygen into the sample unless the length of tubing is minimized and the flow rate is maximized.
- Flush the sampling system with sample for at least 5 minutes.

Accuracy check

The reagent blank for this test can be checked by following these steps:

1. Fill a 50-mL beaker with sample and add one sodium hydrosulfite powder pillow.
2. Immerse the tip of a Low Range Dissolved Oxygen AccuVac Ampul in the sample into the tip. Aspirate the sample into the Ampul.
3. Determine the dissolved oxygen concentration according to the preceding procedure. The result should be 0 ± 6 µg/L.

Method performance

Sensitivity

Program	Sensitivity—ΔConcentration per 0.010 ΔAbs
446	6 µg/L O_2

Summary of method

The Low Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum-sealed in an Ampul. When the AccuVac Ampul is broken open in a sample containing dissolved oxygen, the yellow solution will turn blue. The blue color development is proportional to the concentration of dissolved oxygen. Test results are measured at 610 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Low Range Dissolved Oxygen AccuVac® Ampuls	1	25/pkg	2501025

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Polypropylene Beaker, 50-mL	1	each	108041
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Hydrosulfite reagent powder pillows	100/pkg	2118869

Optional reagents, apparatus and meters

Description	Unit	Catalog number
AccuVac® snapper	each	2405200
AccuVac sampler	each	2405100
AccuVac, vials for sample blanks	25/pkg	2677925
AccuVac stoppers	6/pkg	173106

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Edition 7

Oxygen, Dissolved

DOC316.53.01097

Ultra High Range Method **Method 8333**

UHR (1.0 to 40.0 mg/L O_2) **AccuVac® Ampuls**

Scope and Application: For aquaculture

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 282 Instrument-specific information

Instrument	AccuVac Ampuls	
	Sample cell	Adapter
DR 6000	2427606	—
DR 5000	2427606	—
DR 3900	2427606	LZV846 (A)
DR 3800, DR 2700, DR 2800	2122800	LZV584 (C)

Before starting the test:

Analyze samples on-site. Do not store for later analysis

Collect the following items:

Description	Quantity
High Range Dissolved Oxygen AccuVac®Ampuls, with reusable Ampul caps	1
Polypropylene Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1

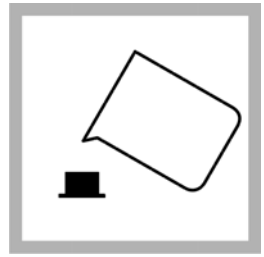
See *Consumables and replacement items* for reorder information.

UHR Method for AccuVac® Ampuls

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. Blank Preparation: Fill a sample cell with 10 mL of sample.

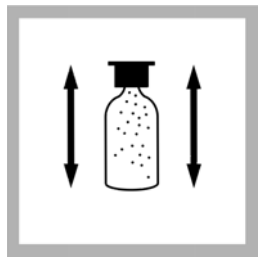


3. Fill a blue Ampul cap with sample.

4. Prepared Sample: Fill a High Range Dissolved Oxygen AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

5. Hold the Ampul with the tip pointing down and immediately insert the Ampul into the Ampul cap.

The cap prevents contamination from atmospheric oxygen.

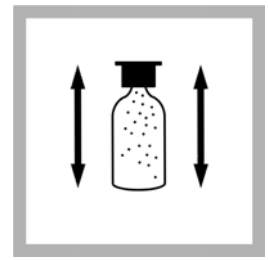


6. Shake the Ampul for 30 seconds.

A small amount of undissolved reagent will not affect results.

7. Start the instrument timer.

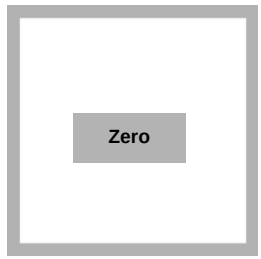
A two-minute reaction period will begin. This enables the oxygen that was degassed during aspiration to redissolve and react.



8. When the timer expires, shake the Ampul for 30 seconds.

Allow any bubbles to dissipate before proceeding.

9. Insert the blank in the cell holder.

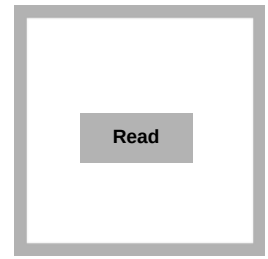


10. ZERO the instrument.

The display will show:

0.0 mg/L O_2

11. Insert the prepared sample into the cell holder.



12. READ the results in mg/L O_2.

Interferences

Table 283 Interfering substances

Interfering substance	Interference level
Cr^{3+}	Greater than 10 mg/L
Cu^{2+}	Greater than 10 mg/L
Fe^{2+}	Greater than 10 mg/L
Mg^{2+}	Magnesium is commonly present in seawater and causes a negative interference. If the sample contains more than 50% seawater, the oxygen concentration obtained by this method will be 25% less than the true oxygen concentration. If the sample contains less than 50% seawater, the interference will be less than 5%.
Mn^{2+}	Greater than 10 mg/L
Ni^{2+}	Greater than 10 mg/L
NO_2^-	Greater than 10 mg/L

Sample collection, preservation and storage

The main consideration in sampling with the High Range Dissolved Oxygen Ampul is to prevent the sample from becoming contaminated with atmospheric oxygen between breaking open the Ampul and reading the absorbance. This is accomplished by capping the Ampul with an Ampul cap. If the Ampul is securely capped, the Ampul should be safe from contamination for several hours. The absorbance will decrease by approximately 3% during the first hour and will not change significantly afterwards.

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested may change with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time and other factors. A single dissolved oxygen test rarely reflects the accurate overall condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results. Samples must be tested immediately upon collection, although only a small error results if the absorbance reading is taken several hours later.

Accuracy check

The results of this procedure may be compared with the results of a titrimetric procedure (request Lit. Code 8042) or by using a dissolved oxygen meter.*

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
448	26.4 mg/L O_2	23.6–29.2 mg/L O_2	0.34 mg/L O_2
			0.45 mg/L O_2
			0.68 mg/L O_2

* See *Optional reagents, apparatus and meters*.

Summary of method

The High Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum-sealed in an Ampul. When the AccuVac Ampul is opened in a sample containing dissolved oxygen, it forms a yellow color which turns purple. The purple color development is proportional to the concentration of dissolved oxygen. Test results are measured at 680 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
High Range Dissolved Oxygen AccuVac® Ampuls with 2 reusable Ampul caps	1	25/pkg	2515025

Required apparatus (AccuVac)

Description	Quantity	Unit	Catalog number
Polypropylene Beaker, 50-mL	1	each	108041
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional reagents, apparatus and meters

Description	Unit	Catalog number
AccuVac® snapper	each	2405200
AccuVac sampler	each	2405100
AccuVac, vials for sample blanks	25/pkg	2677925
AccuVac stoppers	6/pkg	173106
HQ30d Meter with Standard LDO Dissolved Oxygen Probe (1 meter cable)[1]	each	HQ30d53301000
HQ40d Meter with Standard LDO Probe (1 meter cable)[1]	each	HQ40d53301000

[1] Additional probes and cable lengths are available.

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Edition 7

Oxygen, Dissolved

DOC316.53.01179

Azide Modification of Winkler Method

Method 8215 and 8332

1 to greater than 10 mg/L

Digital Titrator

Scope and Application: For water, wastewater and seawater

Test preparation

Before starting the test:

Use the TitraStir apparatus for best results.

Collect the following items:

Description	Quantity
Dissolved Oxygen Reagent Set	1
Sodium Thiosulfate Titration Cartridge, 2.00 N	varies
Bottle, with stopper, BOD, 300-mL	1
Clippers, for opening pillows	1
Cylinder, graduated, 250-mL	1
Digital Titrator	1
Flask, Erlenmeyer, 250-mL	1
Dissolved Oxygen 1 Reagent Powder Pillows	1
Dissolved Oxygen 2 Reagent Powder Pillows	1
Dissolved Oxygen 3 Reagent Powder Pillows	1
Sodium Thiosulfate Titration Cartridge, 0.2000 N	1
Bottle, with stopper, BOD, 60-mL	1
Flask, Erlenmeyer, 125-mL	1
Polypropylene Beaker, 50-mL, Low Form, with pour spout	1

See *Consumables and replacement items* for reorder information.

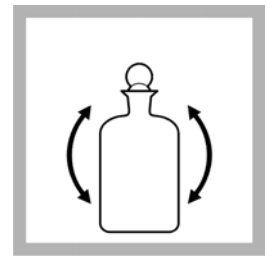
Method 8215, 300-mL BOD bottle

1. Collect a water sample in a clean 300-mL BOD bottle. Allow the sample to overflow the bottle for 2–3 minutes to make sure that a representative sample is available.

2. Add the contents of one Manganous Sulfate Powder Pillow and one Alkaline Iodide-Azide Reagent Powder Pillow.

3. Immediately and without trapping air in the bottle, insert the stopper. Invert the bottle several times to mix.

A flocculent precipitate will form. It will be orange-brown if oxygen is present or white if oxygen is absent. The floc settles slowly in salt water. The settling normally takes about five minutes. When the floc settles, proceed to step 4.

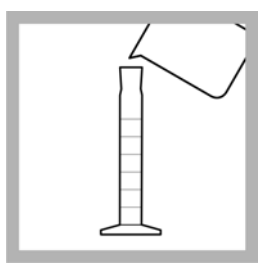


4. Again, invert the bottle several times and wait until the floc settles and the top half of the solution is clear again.

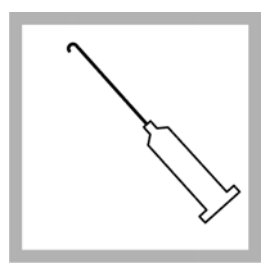
Wait until the floc settles the second time to make sure the reaction of the sample and reagents is complete.

5. Remove the stopper and add the contents of one Sulfamic Acid Powder Pillow. Replace the stopper without trapping air in the bottle. Invert the prepared sample several times to mix.

The floc will dissolve and leave a yellow color if oxygen is present.



6. Select a sample volume and Sodium Thiosulfate Titration Cartridge from the *Volume multipliers* table that corresponds to the expected dissolved oxygen (DO) concentration.



7. Insert a clean delivery tube into the titration cartridge.

Attach the cartridge to the titrator body.

8. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

Method 8215, 300-mL BOD bottle (continued)

9. Use a graduated cylinder to measure the sample volume from the *Volume multipliers* table. Transfer the sample into a 250-mL Erlenmeyer flask.

10. Place the delivery tube tip into the solution and swirl the flask while titrating with sodium thiosulfate to a pale yellow color.

11. Add two 1-mL droppers of Starch Indicator Solution and swirl to mix.

A dark blue color will develop.

12. Continue the titration to a colorless end point. Record the number of digits required.

13. Calculate:
Digits Required x
Digit Multiplier =
mg/L Dissolved Oxygen

Table 284 Volume multipliers

Range (mg/L DO)	Volume (mL)	Titration Cartridge (N $Na_2S_2O_3$)	Digit Multiplier
100–400	150	0.200	0.01
200–800	75	0.200	0.02
600–2400	25	2.000	0.10

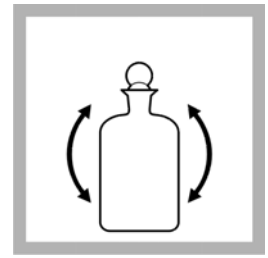
Method 8332, 60-mL BOD bottle

1. Collect a water sample in a clean 60-mL BOD bottle. Allow the sample to overflow the bottle for 2–3 minutes to make sure that a representative sample is obtained.

2. Add the contents of one Dissolved Oxygen 1 Powder Pillow and one Dissolved Oxygen 2 Powder Pillow.

3. Immediately and without trapping air in the bottle, insert the stopper. Invert the bottle several times to mix.

A flocculent precipitate will form. It will be orange-brown if oxygen is present or white if oxygen is absent. The floc settles slowly in salt water. The settling normally takes about five minutes. When the floc settles, proceed to step 4.



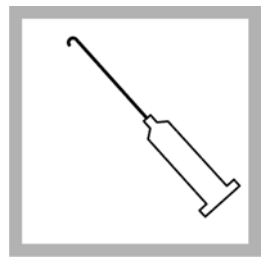
4. Again invert the bottle several times and wait until the floc settles and the top half of the solution is clear again.

Wait until the floc settles the second time to make sure the reaction of the sample and reagents is complete.

5. Remove the stopper and add the contents of one Dissolved Oxygen 3 Powder Pillow. Replace the stopper without trapping air in the bottle and invert several times to mix.

The floc will dissolve and leave a yellow color if oxygen is present.

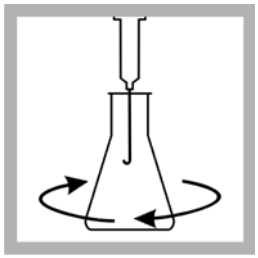
6. Accurately measure 20 mL of the prepared sample and transfer it to a 50-mL Erlenmeyer flask.



7. Insert a clean straight-stem delivery tube to a 0.200 N Sodium Thiosulfate Titration Cartridge. Attach the cartridge onto the titrator body.

8. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

Method 8332, 60-mL BOD bottle (continued)



9. Titrate the prepared solution with 0.2000 N Sodium Thiosulfate until the sample changes to a pale yellow color. Record the number of digits.

10. Add two 1-mL droppers of Starch Indicator Solution and swirl to mix.

A dark blue color will develop.

11. Continue the titration to a colorless end point. Record the number of digits required.

12. Calculate:
Digits Required x 0.1 = mg/L Dissolved Oxygen

Interferences

Nitrite interference is eliminated by the azide in the reagents. Other reducing or oxidizing substances may interfere. If these are present, use an alternate method, such as the High Range Dissolved Oxygen Method (colorimetric, Method 8166) or a dissolved oxygen electrode (4500 OG).

Sample collection, preservation and storage

Sampling and sample handling are important in obtaining meaningful results. The dissolved oxygen content of the sample changes with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time and other factors. A single dissolved oxygen test rarely reflects the over-all condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results.

- Collect samples in clean BOD Bottles.
- If storage is necessary, run steps 1 through 4 of the procedure and store in the dark at 10–20 °C.
- Seal the bottle with water by pouring a small volume of water into the flared lip area of a stopper bottle.
- Use a BOD Bottle Cap over the flared lip.
- Samples preserved like this can be held 4–8 hours. Begin with step 5 when analyzing.

Accuracy check

Check the strength of the Sodium Thiosulfate Solution by using an Iodate-Iodide Standard Solution, 10 mg/L as DO.

1. For the 300-mL procedure, begin at step 5, adding the Sulfamic Acid Powder Pillow to a 200-mL volume of Iodate-Iodide Standard Solution.
2. Use a 100-mL sample volume with the 0.200 N Sodium Thiosulfate Titration Cartridge. This titration should take 500 digits. If more than 525 digits are required to reach the end point, replace the Sodium Thiosulfate Cartridge.

3. Use a 200-mL sample volume with the 2.00 N Sodium Thiosulfate Titration Cartridge. This titration should take 100 digits. If more than 105 digits are required to reach the end point, replace the Sodium Thiosulfate Cartridge.

For the 60-mg/L procedure:

1. Begin the analysis at step 5, adding the Dissolved Oxygen 3 Powder Pillow to a 60 mL volume of Iodate-Iodide Standard Solution.
2. Use a 20 mL sample volume with the 2.00 N Sodium Thiosulfate Titration Cartridge. This titration should take 100 digits. If more than 105 digits are required to reach the end point, replace the Sodium Thiosulfate Cartridge.

Summary of method

Samples are treated with manganous sulfate and alkaline iodide-azide reagent to form an orange-brown precipitate. Upon acidification of the sample, this floc reacts with iodide to produce free iodine as triiodide in proportion to the oxygen concentration. The iodine is titrated with sodium thiosulfate to a colorless end point.

Consumables and replacement items

Required reagents for 300-mL BOD bottle

Description	Unit	Catalog number
Dissolved Oxygen Reagent Set (about 50 tests)		2272200
Includes:		
(2) Alkaline Iodide-Azide Powder Pillows	50/pkg	107266
(2) Manganous Sulfate Powder Pillows	50/pkg	107166
(1) Sodium Thiosulfate Titration Cartridge, 0.2000 N	each	2267501
(1) Starch Indicator Solution	100 mL MDB[1]	34932
(2) Sulfamic Acid Powder Pillows	50/pkg	2076266
Sodium Thiosulfate Titration Cartridge. 2.00 N	each	1440101

[1] MDB is Marked Dropper Bottle

Required apparatus for 300-mL BOD bottle

Description	Unit	Catalog number
Bottle, with stopper, BOD, 300-mL	each	62100
Clippers, for opening pillows	each	96800
Cylinder, graduated, 250-mL	each	50846
Digital Titrator	each	1690001
Flask, Erlenmeyer, 250-mL	each	50546
Delivery tubes w/ 180° hook	each	1720500
Delivery tubes w/ 90° hook	each	4157800

Required reagents for 60-mL BOD bottle

Description	Unit	Catalog number
Dissolved Oxygen 1 Reagent Powder Pillows	100/pkg	98199
Dissolved Oxygen 2 Reagent Powder Pillows	100/pkg	98299
Dissolved Oxygen 3 Reagent Powder Pillows	25/pkg	98768
Sodium Thiosulfate Titration Cartridge, 0.2000 N	each	2267501

Required apparatus for 60-mL BOD bottle

Description	Unit	Catalog number
Bottle, with stopper, BOD, 60-mL	each	190902
Clippers, for opening pillows	each	96800
Digital Titrator	each	1690001
Flask, Erlenmeyer, 125-mL	each	50543
Polypropylene Beaker, 50-mL, Low Form, with pour spout	each	108041
Delivery tubes w/ 180° hook	each	1720500
Delivery tubes w/ 90° hook	each	4157800

Required standards

Iodate-Iodide Standard Solution, 10-mg/L as DO	500 mL	40149
Thermometer -10 –225 °C 405 mm	each	2635700
Cap, BOD Bottle Snap-over	6/pkg	241906
BOD Bottle, Serialized (#1-24)	24/pkg	2898700

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Edition 7

Oxygen, Dissolved

DOC316.53.01161

USEPA[1] Azide Modification of Winkler Method[2]

Method 8229

1 to more than 10 mg/L DO

Buret Titration

Scope and Application: For water, wastewater and seawater.

[1] USEPA approved.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater,* (*Standard Method* 4500 O C)

Test preparation

Before starting the test:

Dissolved oxygen can be lost from the sample during sample collection. Review the precautions in *Sample collection, preservation and storage* before the test is started.

Standard APHA solutions for dissolved oxygen can be used in place of the powder pillow reagents by substituting 1 mL of Manganous Sulfate Solution, 1 mL of Alkaline Iodide-Azide Reagent and 1 mL of Sulfuric Acid (concentrated) in place of the powder pillows. These solutions must be dispensed below the surface of the liquid.

Collect the following items:

Description	Quantity
BOD bottle, 300-mL	1
Alkaline Iodide-Azide Reagent Powder Pillow	1 pillow
Manganous Sulfate Powder Pillow	1 pillow
Sulfamic Acid Powder Pillow	1 pillow
Sodium Thiosulfate Standard Solution (titrant), 0.025 N	1 bottle
Starch Indicator Solution	1 bottle
Buret, Class A, 25-mL, with support stand	1
Graduated cylinder, 250-mL	1
Erlenmeyer flask, 250-mL	1

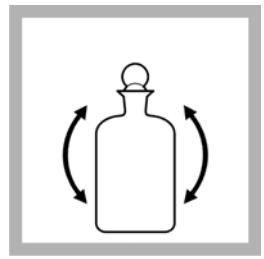
See *Consumables and replacement items* for reorder information.

Buret titration

1. Collect a water sample in a clean, 300-mL, glass stoppered BOD bottle.

Overflow the bottle for two or three minutes to remove any trapped air bubbles to make sure that a representative sample is available.

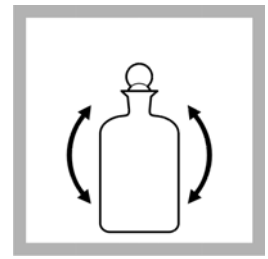
2. Add the contents of one Manganous Sulfate Powder Pillow and one Alkaline Iodide-Azide Reagent Powder Pillow.



3. Immediately insert the stopper so that no air is trapped in the bottle. Invert several times to mix.

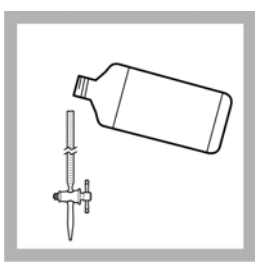
A flocculent precipitate will form. It will be orange-brown if oxygen is present or white if oxygen is absent.

The floc will settle very slowly in salt water. Wait five more minutes before proceeding to step 4.



4. Again invert the bottle several times and wait until the floc has settled and the top half of the solution is clear again.

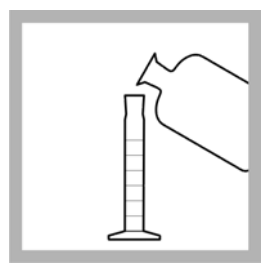
Waiting until floc has settled twice makes sure that the reaction is complete. Results will not be affected if the floc does not completely settle.



5. Fill a 25-mL buret to the zero mark with 0.025 N Sodium Thiosulfate Solution.

6. Remove the stopper and add the contents of one Sulfamic Acid Powder Pillow. Replace the stopper without trapping air in the bottle and invert several times to mix. This is the prepared sample.

The floc will dissolve and leave a yellow color if oxygen is present.



7. Pour the prepared sample into a 250-mL graduated cylinder to the 200-mL mark.

8. Pour the contents of the graduated cylinder into a 250-mL Erlenmeyer flask.

Buret titration (continued)

9. Titrate the sample while gently swirling the flask until it turns a very pale yellow color.

10. Add two 1-mL droppers of Starch Indicator Solution. Swirl to mix.

The solution will turn dark blue.

11. Continue the titration until the solution changes from dark blue to colorless.

The amount of titrant used to reach the end point is equal to the concentration of dissolved oxygen in the sample.

mL titrant used = mg/L DO

Interferences

Nitrite interference is eliminated by the azide in the reagents. Other reducing or oxidizing substances may interfere. If these are present, use an alternate method, such as the High Range Dissolved Oxygen Method, (Hach method 8166 - colorimetric) or a dissolved oxygen electrode (Standard Method 4500 O G).

Pretreatment procedure for activated sludge samples
A sample pretreatment is necessary for activated sludge samples.

1. Add 10 mL of Copper Sulfate-Sulfamic Acid Inhibitor Solution to a clean 1000-mL graduated cylinder.
2. Fill the cylinder with the sample using a tube that empties near the bottom of the cylinder and allow the sample to overflow by about 200 mL.
3. Swirl the cylinder to mix the contents. Allow the suspended solids to settle.
4. Siphon the relatively clear top layer into a BOD bottle through a siphon tube extended to the bottom of the bottle. Withdraw the siphon tube while the water is flowing. Make sure that no air bubbles are trapped in the bottle. Continue with step 2–step 11 of the test procedure.

Sample collection, preservation and storage

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested varies with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time and other factors. A single dissolved oxygen test rarely reflects the overall condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results.

Collect samples in a clean BOD bottle as described in step 1. If storage is necessary, complete steps 1–4 of the test procedure and store in the dark at the temperature of the water source or water seal at 10–20 °C. (Sealing with water is done by pouring a small amount of water into the

flared lip area of a stoppered bottle. Snap a BOD bottle cap over the flared lip). Samples preserved in this manner can be held four to eight hours. Start the test at step 6.

Accuracy check

The standard solution method can be used to confirm analytical technique and reagent performance.

Standard solution method
Complete the following test to make sure the concentration of the titrant is accurate.

Required for accuracy check:

- Iodate-Iodide Standard Solution, 0.00125 N (equivalent to 10 mg/L as O_2)

1. Add 200.0 mL of Iodate-Iodide Standard Solution, 0.00125 N, to an Erlenmeyer flask.
2. Add one Sulfamic Acid Powder Pillow and swirl to mix.
3. Follow steps 9–11 of the test procedure to titrate the standard to the end point. The titration should use 10.0 mL of the titrant solution. If more than 10.5 mL is used, discard the titrant and replace it with a fresh supply.

Summary of method

The Azide Modification of the Winkler Method is the standard test for dissolved oxygen. In the analysis, manganous ion reacts with the dissolved oxygen present in the alkaline solution to form a manganese (IV) oxide hydroxide flocculent. Azide is then added to suppress interference from any nitrite, which would react with the iodide. The solution is then acidified and the manganese (IV) floc is reduced by iodide to produce free iodine as I_3^- in proportion to the oxygen concentration. The liberated iodine is then titrated to the starch-iodide end point.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Alkaline Iodide-Azide Reagent Powder Pillows	1 pillow	50/pkg	107266
Manganous Sulfate Powder Pillows	1 pillow	50/pkg	107166
Sodium Thiosulfate Standard Solution (titrant), 0.025 N	varies	1 L	2409353
Starch Indicator Solution	2 mL	100 mL MDB	34932
Sulfamic Acid Powder Pillows	1 pillow	100/pkg	107399

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Bottle, glass-stoppered, BOD, 300-mL	1	each	62100
Buret, Class A, 25-mL	1	each	2636540
Buret Clamp, double	1	each	32800
Clippers, for opening powder pillows	1	each	96800
Cylinder, graduated, 250-mL	1	each	50846
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Support Stand	1	each	56300

Recommended standards

Description	Unit	Catalog number
Iodate-Iodide Standard Solution, 0.00125 N	500 mL	40149

Optional reagents and apparatus

Description	Unit	Catalog number
APHA reagents:		
Alkaline Iodide-Azide Reagent Solution	500 mL	27749
Manganous Sulfate Solution	500 mL	27549
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	35253
Sulfuric Acid, ACS	500 mL	97949
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Graduated cylinder, 1000 mL	each	50853
BOD bottle caps	6/pkg	241906
BOD bottles, serialized	24/pkg	2898700
Copper Sulfate-Sulfamic acid inhibitor	100 mL	35732
Copper Sulfate-Sulfamic acid inhibitor	500 mL	35749

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Edition 7

Oxygen Scavengers

DOC316.53.01105

Iron Reduction Method for Oxygen Scavengers

Method 8140

5 to 600 µg/L carbohydrazide;
3 to 450 µg/L DEHA;
9 to 1000 µg/L hydroquinone;
13 to 1500 µg/L iso-ascorbic acid [ISA];
15 to 1000 µg/L methylethyl ketoxime [MEKO]

Powder Pillows

Scope and Application: For testing residual corrosion inhibitors (oxygen scavengers) in boiler feed water or condensate

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 285 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Analyze samples immediately. Do not preserve for later analysis.
The sample temperature should be 25 ± 3 °C (77 ± 5 °F).
Soak glassware with 1:1 hydrochloric acid solution. Rinse several times with deionized water. These two steps will remove iron deposits that can cause slightly high results.
To determine ferrous iron concentration, repeat the procedure, but do not add DEHA Reagent 2. Correct for the ferrous iron concentration: **OPTIONS>MORE>REAGENT BLANK>ON**. The reading attributed to the ferrous iron concentration will appear.

Collect the following items:

Description	Quantity
Bottle, glass mixing, with 25-mL mark	2
DEHA Reagent 1 Powder Pillow	2
DEHA Reagent 2 Solution	1 mL
Deionized Water	25 mL
Dropper, 0.5 and 1.0 mL marks	1
Hydrochloric Acid, 1:1, 6.0 N	varies
Sample Cells, 1-inch, 10-mL	2

See *Consumables and replacement items* for reorder information.

Iron reduction method for Oxygen Scavengers

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. **Prepared Sample:** Fill a mixing bottle with 25 mL of sample.

When determining oxygen scavengers that react quickly with oxygen at room temperature, cap the bottle.

3. **Blank Preparation:** Fill a second mixing bottle with 25 mL of deionized water.

4. Add the contents of one DEHA Reagent 1 Powder Pillow to each mixing bottle. Swirl to mix.

Iron reduction method for Oxygen Scavengers (continued)

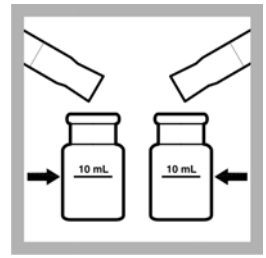
5. Add 0.5 mL of DEHA Reagent 2 Solution to each bottle. Mix. Place both sample cells in the dark.

A purple color will develop if an oxygen scavenger is present.

6. Start the instrument timer.

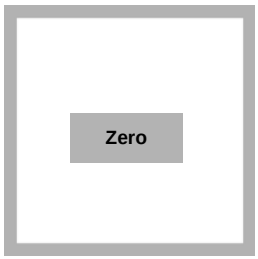
A ten-minute reaction period (or a **two-minute** reaction period for **hydroquinone**) will begin.

Keep the sample cells in the dark during the reaction period.



7. When the timer expires, transfer the blank and prepared samples into the 10-mL sample cells.

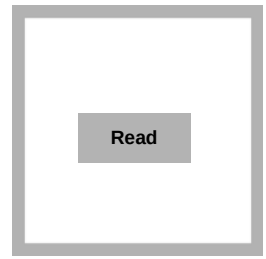
8. Immediately after transferring to the 10-mL cell, wipe the blank and insert it into the cell holder. Close the cover.



9. **ZERO** the instrument.

For greater accuracy, read the result immediately after the timer expires.

10. Immediately wipe the prepared sample and insert it into the cell holder.



11. **READ** the results in µg/L.

Interferences

Substances which reduce ferric iron will interfere. Substances which complex iron strongly may also interfere.

Table 286 Interfering substances

Interfering substance	Interference level
Borate (as $Na_2B_4O_7$)	Greater than 500 mg/L
Cobalt	Greater than 0.025 mg/L
Copper	Greater than 8.0 mg/L
Ferrous Iron	All levels. ***Note:*** *Determine and subtract (see Before starting the test:)*
Hardness (as $CaCO_3$)	Greater than 1000 mg/L

Table 286 Interfering substances (continued)

Interfering substance	Interference level
Light	Light may interfere. Keep sample cells in the dark during color development.
Lignosulfonates	Greater than 0.05 mg/L
Manganese	Greater than 0.8 mg/L
Molybdenum	Greater than 80 mg/L
Nickel	Greater than 0.8 mg/L
Phosphate	Greater than 10 mg/L
Phosphonates	Greater than 10 mg/L
Sulfate	Greater than 1000 mg/L
Temperature	Sample temperatures below 22 °C or above 28 °C (72 °F or 82 °F) may affect test accuracy.
Zinc	Greater than 50 mg/L

Sample collection, preservation and storage

- Collect samples in clean, dry, plastic or glass containers.
- Avoid excessive agitation or exposure to sunlight when sampling.
- Rinse the container several times with the sample prior to collection.
- Allow the container to overflow and cap the container so that there is no headspace above the sample.
- Rinse the sample cell several times with the reacted sample, then carefully fill to the 10-mL mark. Perform the analysis immediately.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity ΔConcentration change per 0.010 ΔAbs change
180	299 µg/L	295–303 µg/L	4 µg/L
181	226 µg/L	223–229 µg/L	3 µg/L
182	600 µg/L	591–609 µg/L	8 µg/L
183	886 µg/L	873–899 µg/L	12 µg/L
184	976 µg/L	962–990 µg/L	14 µg/L

Summary of method

Diethylhydroxylamine (DEHA) or other oxygen scavengers present in the sample react with ferric iron in DEHA Reagent 2 Solution to produce ferrous ion in an amount equivalent to the DEHA concentration. This solution then reacts with DEHA 1 Reagent, which forms a purple color with ferrous iron proportional to the concentration of oxygen scavenger. Test results are measured at 562 nm. This method reacts with all oxygen scavengers and does not differentiate samples containing more than one type of oxygen scavenger.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Oxygen Scavenger Reagent Set, includes:	—	—	2446600
(2) DEHA Reagent 1 Powder Pillows	2	100/pkg	2167969
(1) DEHA Reagent 2 Solution	1 mL	100 mL	2168042
Hydrochloric Acid, 1:1, 6.0 N	varies	500 mL	88449
Water, deionized	25 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Bottle, glass mixing, with 25-mL mark	2	each	1704200
Dropper, 0.5 and 1.0-mL marks	1	20/pkg	2124720
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional apparatus

Description	Unit	Catalog number
Thermometer, Non-Mercury, –10 to 225 °C	each	2635700

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Edition 7

Indigo Method **Method 8311**

LR (0.01 to 0.25 mg/L O_3),
MR (0.01 to 0.75 mg/L O_3),
HR (0.01 to 1.50 mg/L O_3) **AccuVac® Ampul**

Scope and Application: For water

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 287 Instrument-specific information

Instrument	Adapter
DR 6000	—
DR 5000	—
DR 3900	LZV846 (A)
DR 3800, DR 2800, DR 2700	LZV584 (C)

Before starting the test:

Analyze sample immediately. Do not preserve for later analysis.
Use tap water or deionized water for the blank (ozone-free water)
The sequence of measuring the blank and the sample is reversed in this procedure.

Collect the following items:

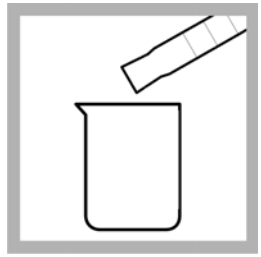
Description	Quantity
Select Ozone AccuVac® Ampuls based on range:	
0–0.25 mg/L	2
0–0.75 mg/L	2
0–1.50 mg/L	2
Beaker, Polypropylene, 50-mL, low form with pour spout	1
Water, ozone-free	varies

See *Consumables and replacement items* for reorder information.

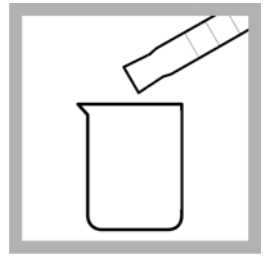
Method Name for powder pillows

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Blank Preparation: Collect at least 40 mL of ozone-free water in a 50-mL beaker.



3. Prepared Sample: Gently collect at least 40 mL of sample in another 50-mL beaker.

4. Fill one Indigo Ozone Reagent AccuVac Ampul with the sample and another with the blank.

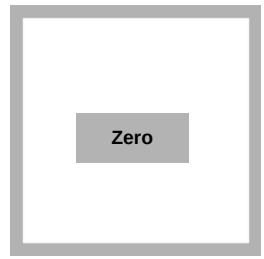
Keep the tip immersed while the Ampul fills.

5. Quickly invert both Ampuls several times to mix.

Some of the blue color will be bleached if ozone is present.

6. Wipe the Ampuls with a cloth to remove fingerprints or other marks.

Insert the **sample** into the cell holder.



7. ZERO the instrument.

The display will show:

0.00 mg/L O_3

8. Insert the **blank** into the cell holder.

Press **READ**. Results are in mg/L O_3.

Sample collection, preservation and storage

The most important consideration when collecting a sample is to prevent the escape of ozone from the sample. The sample should be collected gently and analyzed immediately. Warming the sample or disturbing the sample by stirring or shaking, will result in ozone loss. After collecting the sample, do not transfer it from one container to another unless absolutely necessary.

Stability of Indigo reagent

Because indigo is light-sensitive, the AccuVac Ampuls should be kept in the dark at all times. The indigo solution, however, decomposes slowly under room light after filling with sample. The blank Ampul can be used for multiple measurements during the same day.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity ΔConcentration change per 0.010 ΔAbs change
454	0.15 mg/L	0.14–0.16 mg/L O_3	0.01 mg/L O_3
455	0.45 mg/L	0.43–0.47 mg/L O_3	0.01 mg/L O_3
456	1.00 mg/L	0.97–1.03 mg/L O_3	0.01 mg/L O_3

Summary of method

The reagent formulation adjusts the sample pH to 2.5 after the Ampule has filled. The indigo reagent reacts immediately and quantitatively with ozone. The blue color of indigo is bleached in proportion to the amount of ozone present in the sample. Other reagents in the formulation prevent chlorine interference. No transfer of sample is needed in the procedure, therefore ozone loss due to sampling is eliminated. Test results are measured at 600 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Select one or more Ozone AccuVac® Ampules based on range:			
0–0.25 mg/L	2	25/pkg	2516025
0–0.75 mg/L	2	25/pkg	2517025
0–1.5 mg/L	2	25/pkg	2518025

Required apparatus

Description		Unit	Catalog number
Polypropylene Beaker, 50-mL, Low Form		each	108041
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Optional reagents and apparatus

Description	Unit	Catalog number
Snapper, AccuVac®	each	2405200
Water, demineralized	4 L	27256
SpecCheck Gel Secondary Standard Kit, Ozone, 0 – 0.75 mg/L set	each	2708000
Test tube stopper	6/pkg	173106

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Edition 7

Polychlorinated Biphenyls (PCB) in Soil

DOC356.53.01107

Immunoassay Method[1] **Method 10050**

Scope and Application: For soil

[1] This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 288 Instrument-specific information

Instrument	Adapter
DR 6000	—
DR 5000	A23618
DR 3900	LZV846 (A)
DR 3800, DR 2800, DR 2700	LZV583

Before starting the test:

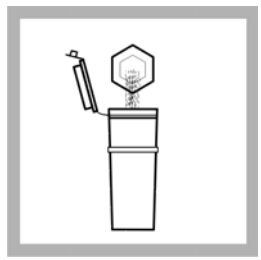
This method analyzes for PCB that has been extracted from soil samples. Sample extracts, calibrators and reagents are added to cuvettes coated with PCB-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 20 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously
Read the entire procedure before starting. Identify and make ready all the necessary reagents, cuvettes and other apparatus before beginning the analysis.
Timing is critical; follow instructions carefully.
A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in *Use the 1-cm MicroCuvette rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
There are two protocols in this procedure, one for levels of 1 ppm and 5 ppm and another for 10 ppm and 50 ppm. Each uses a different quantity of calibrator and sample extract See *PCB protocols* for more information.
Store the reagents at 4 °C when they are not in use. Allow the reagents to reach room temperature before using them in an analysis. Actual testing may be done at temperatures ranging from 1– 38 °C.
The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.
Protective nitrile gloves are recommended for this procedure.

Description	Quantity
PCB Reagent Set	1
Water, deionized	varies
Caps, flip spout	1
Marker, laboratory	1
Rack, for 1-cm Micro Cuvettes	1
Wipes, disposable	1
Pipet, TenSette®, 0.1–1.0 mL and pipet tips	1
Soil Extraction Kit and Soil Scoop	1
Analytical Balance	1
Cylinder, graduated	1
Scoop, 5 g	1

See *Consumables and replacement items* for reorder information.

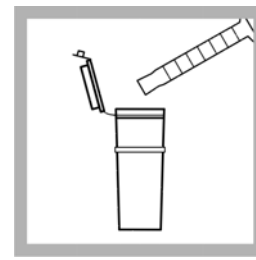
Soil extraction procedure

1. Weigh out 5 g of soil in the plastic weighing boat.



2. Carefully pour the soil into an extraction vial.

3. Use the 5-gram scoop to add one scoop of sodium sulfate to the extraction vial.

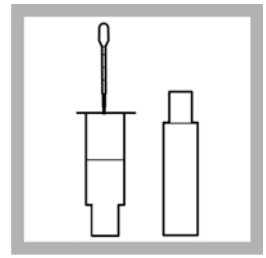


4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial.

Soil extraction procedure

5. Cap the extraction vial tightly and shake vigorously for one minute.

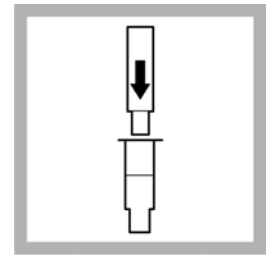
6. Allow to settle for at least one minute. Carefully open the extraction vial.



7. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid layer at the top of the extraction vial.

Transfer it into the filtration barrel (the bottom part of the filtering assembly into which the plunger inserts).

Do not use more than 1.5 mL. The pipet is marked in 0.25-mL increments.

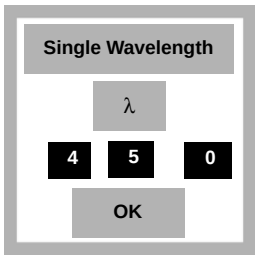


8. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until the sample extract is forced upward into the center of the plunger.

Use the resultant filtrate for the immunoassay in *Immunoassay for soil extracts*.

It may be necessary to place the filtration assembly on a table and press down on the plunger.

Immunoassay for soil extracts



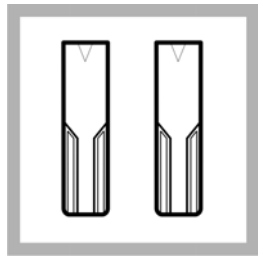
1. Press

SINGLE WAVELENGTH

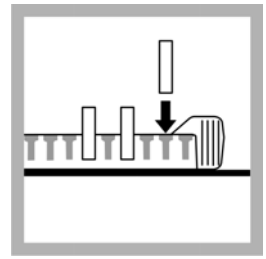
Press **OPTIONS** and the λ button.

Enter 450 nm and press OK.

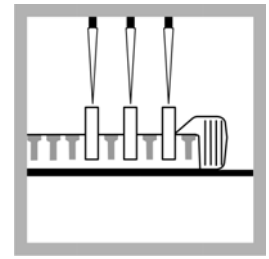
Insert an adapter if required (*Instrument-specific information*). Refer to the user manual for orientation.



2. Label an Antibody Cuvette for each calibrator and each sample to be tested.



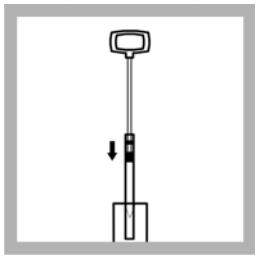
3. Insert the cuvettes into the rack snugly.



4. Pipet 0.5 mL of each diluent solution into each cuvette. The same pipet tip can be used repeatedly for this step.

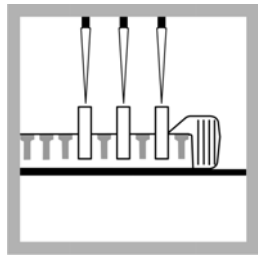
Have the necessary apparatus at hand for the next four steps; they must be done without delay.

Immunoassay for soil extracts (continued)

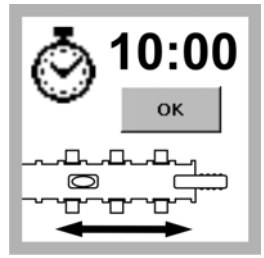


5. Use a Wiretrol® pipet to transfer the appropriate volume of calibrator or sample extract into each cuvette (see the *PCB protocols* table).

Use a separate capillary tube for each solution.



6. Immediately pipet 0.5 mL of PCB Enzyme Conjugate into each calibrator and sample cuvette. The same pipette tip can be used to add the enzyme conjugate to each cuvette.



7. Set the instrument timer for 10:00 minutes. Start the reaction period.

A 10-minute reaction time will begin. Immediately begin mixing the cuvettes for 30 seconds. See *Use the 1-cm MicroCuvette rack*.

8. After 5 minutes mix the contents of the rack for 30 seconds (*Use the 1-cm MicroCuvette rack*.)

9. At the end of the 10-minute period, discard the contents of all the cuvettes into an appropriate waste container.

The sample PCBs and calibrator PCBs remain attached to the cuvette walls.

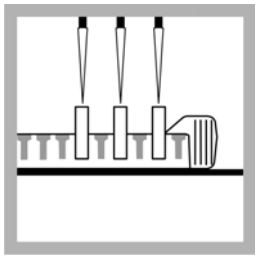
10. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Make sure that most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.

Immunoassay for soil extracts (continued)

Color Development

Important Note: *Timing is critical. Follow instructions carefully.*

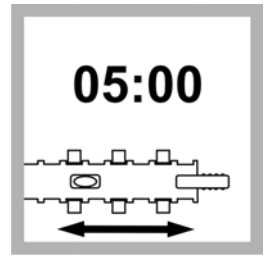


11. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each cuvette.

Use a new pipette tip for each cuvette.

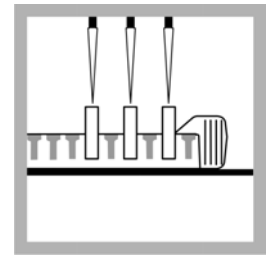
12. Set the instrument timer for 5:00 minutes. Start the reaction period.

Mix, using the instructions in *Use the 1-cm MicroCuvette rack*.



13. After 2.5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique as step 12.

Solutions will turn blue in some or all of the cuvettes.



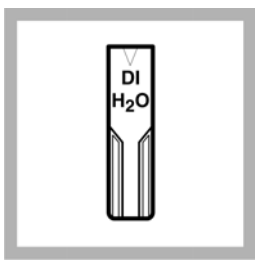
14. At the end of the 5-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 11.

Slide the rack for 20 seconds (see *Use the 1-cm MicroCuvette rack*.)

Blue solutions will turn yellow with the addition of the Stop Solution.

Use the same pipette tip repeatedly for this step.

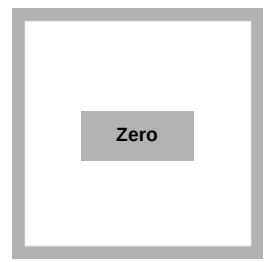
Measuring the Color



15. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges and fingerprints.

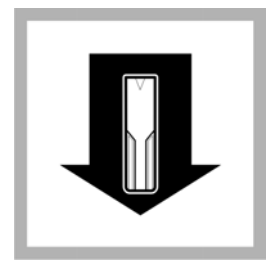
16. Insert the filled Zeroing Cuvette into the cell holder see Table *Instrument-specific information* for cell orientation.

Orient the arrow in the same direction for all cuvettes.



17. **ZERO** the instrument.

The display will show: 0.000 Abs



18. Insert the first calibrator into the cell holder.

Record the results (ABS) for each calibrator and sample.

See *Interpreting and reporting results* for help with interpretation of results.

PCB protocols

There are two protocols in this procedure, one for levels of 1 ppm and 5 ppm and another for 10 ppm and 50 ppm. Each uses a different volume of calibrator and sample extract. Refer to the *PCB protocols* table for range and volume information.

Table 289 PCB protocols

Range (as Arochlor 1248)	Volume of calibrator and sample extract used
1 ppm and 5 ppm	50 µL
10 ppm and 50 ppm	10 µL

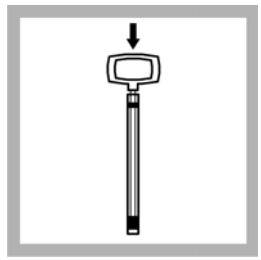
To test across ranges, such as 1 and 50 ppm, test the lower concentration first. If the result is positive then test at the higher level. If the result of the test at the lower concentration is negative, the higher range test will be negative also and need not be performed.

The same filtered extract can be used for both protocols if it is tightly capped between assays. The maximum time between assays cannot exceed one-half hour.

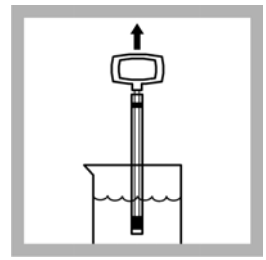
Using the Wiretrol®* pipet

The Wiretrol Pipet can accurately measure small quantities of liquids. It consists of two parts: a Teflon®-tipped plunger and a calibrated capillary tube. The plunger can be reused; the capillary tubes must be discarded after one use.

1. Wet the orange Teflon® tip of the Wiretrol plunger in the sample and carefully insert it into the end of the capillary tube with the colored band.

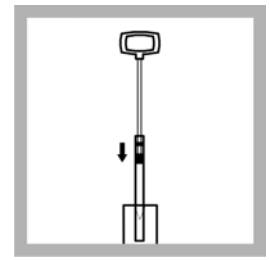


2. Push the tip to the other end of the capillary tube until it barely extends beyond the end of the capillary tube.



3. Submerge the capillary tube below the surface of the liquid to be pipetted. Slowly and smoothly draw the Wiretrol plunger up until the bottom of the plunger tips reaches the appropriate volume line.

Touch the end of the tube to the side of the vessel to release remaining drops on the capillary tube tip.



4. To discharge the pipet, place the tip of the capillary tube **below the surface of the solution** and push the Wiretrol plunger down in one smooth motion. Change capillary tubes for each calibrator and sample.

* Wiretrol is a registered trademark of Drummond Scientific.

Use the 1-cm MicroCuvette rack

The MicroCuvette rack (refer to *The 1-cm MicroCuvette rack* figure) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

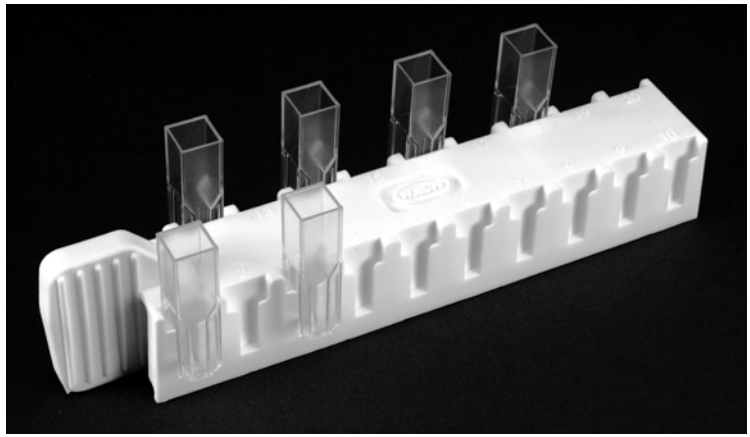


Figure 1 The 1-cm MicroCuvette rack

Loading the rack—The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and insert all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing—Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and reporting results

There is an inverse relationship between the concentration of PCB and the reading. In other words, the higher the reading, the lower the concentration of PCB.

Table 290 Relative PCB Concentration

If the sample reading is...	the sample PCB Concentration is...
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example

Readings:

1 ppb PCB Calibrator: **0.775 Abs**

5 ppb PCB Calibrator: **0.430 Abs**

Sample #1: **0.200 Abs**

Sample #2: **0.600 Abs**

Sample #3: **0.900 Abs**

Interpretation for a soil sample

Sample #1—Sample reading is less than the readings for both calibrators. Therefore the sample concentration of PCB is greater than both 1 ppm and 5 ppm as Aroclor 1248.

Sample #2—Sample reading is between the readings for the 1 ppm and 5 ppm PCB calibrators. Therefore the sample concentration of PCB is between 1 ppm and 5 ppm as Aroclor 1248.

Sample #3—Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of PCB is less than both 5 ppm and 1 ppm as Aroclor 1248.

Storing and handling reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The PCB immunoassay cannot differentiate between the various Arochlors, but it detects their presence in differing degrees.

Table 291 Various PCBs in soil

Compound	Concentration (ppm) to give a positive result at			
	1 ppm	5 ppm	10 ppm	50 ppm
1248	1	5	10	50
1016	2	9	20	67
1242	1.2	6	14	50
1254	1.4	4.6	11	28
1260	1.1	4.9	11	38

Table 292 Compounds not detectable at 1000 ppm

Biphenyl	2,4,6-trichlorophenyl	1,3-dichlorobenzene
2,4-dichlorophenyl	pentachlorophenol	1,4-dichlorobenzene
2,4,5-trichlorphenyl	1,2-dichlorobenzene	1,2,4-trichlorobenzene

Sample collection and storage

- Analyze the samples as soon as possible after collection.
- If the samples must be stored, collect them in glass or Teflon® containers that have been washed with soap and water and rinsed with methanol. The container should be capped with a Teflon-lined cap.
- If a Teflon cap is not available, aluminum foil rinsed in methanol may be used as a substitute cap liner.

Summary of method

Immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Antibodies specific for PCB are attached to the walls of plastic cuvettes. They selectively bind and remove PCB from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and PCB compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by PCB and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of PCB in the sample. The resulting color is then compared with a calibrator to determine whether the PCB concentration in the sample is greater or less than the threshold levels. The PCB concentration is inversely proportional to the color development: the lighter the color, the higher the PCB concentration. Test results are measured at 450 nm.

The method reacts with all PCBs and cannot differentiate samples containing more than one type of PCB.

Consumables and replacement items

Required reagents

Description	Unit	Catalog Number
PCB Reagent Set[1]	20 cuvettes	2773500
Deionized Water	500 mL	27249

[1] Immunoassay components are manufactured by Beacon Analytical Systems, Inc.

Required apparatus

Description	Unit	Catalog Number
Caps, flip spout	2/pkg	2581802
Marker, laboratory	each	2092000
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Rack, for 1-cm Micro Cuvettes	each	4879900
Wipes, disposable	280/box	2097000
Balance, analytical, 80 g capacity, 100–240 VAC	each	2936701
Graduated cylinder, 10-mL	each	108138
Soil Scoop, 5-g, 4.25-cc	each	2657205
Soil Extraction Refill Kit, includes:	each	2775200
Dropper, LDPE, 0.5 and 1.0-mL	20/pkg	2124720
Filter and Barrel Assembly	20/pkg	2567620
Sodium Sulfate, anhydrous	250 g	709929
Soil Extractant Solution	200 mL	2567729
Soil Sample Container	20/pkg	2592920
Weighing Boat, 8.9-cm square	20/pkg	2179020
Spatula, disposable	2/pkg	2569320

Optional reagents and apparatus

Description	Unit	Catalog Number
Gloves, Disposable, Nitrile, Medium[1]	100/pkg	2550502
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	2185696
Goggles, Safety vented	each	2550700

[1] Other sizes are available.

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Edition 7

Phenols

DOC316.53.01108

USEPA[1] 4-Aminoantipyrine Method[2] **Method 8047**

0.002 to 0.200 mg/L

Scope and Application: For Water and Wastewater

[1] USEPA accepted (distillation required); procedure is equivalent to USEPA method 420.1 for wastewater

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 293 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2612602	Fill line faces right
DR 5000	2612602	Fill line faces user
DR 3900	2612602	Fill line faces user
DR 3800, DR 2800, DR 2700	2612602	Fill line faces right

Before starting the test:

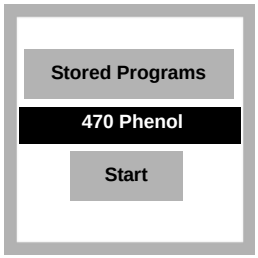
Analyze samples within four hours to avoid oxidation.
Spilled reagent affects test results and is hazardous to skin and other materials.
Use chloroform only with proper ventilation.
Phenol 2 Reagent Powder Pillows contain potassium ferricyanide. Both chloroform (D022) and cyanide (D001) solutions are regulated as hazardous waste by the Federal RCRA. **Do not pour these materials down the drain.** Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent release of hydrogen cyanide gas. Refer to a current MSDS for safe handling and disposal information.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

Collect the following items:

Description	Quantity
Chloroform, ACS	60 mL
Clippers	1
Cotton Balls	1
Cylinder, graduated, 50-mL	1
Cylinder, graduated, 500-mL	1
Funnel, separatory, 500-mL	2
Hardness 1 Buffer Solution, pH 10.1	10 mL
Phenol 2 Reagent Powder Pillows	2
Phenol Reagent Powder Pillows	2
Pipet, volumetric, Class A, 5.00-mL	1
Ring, support, 4-inch	2
Sample Cells (see *Instrument-specific information*)	1
Support for Ring Stand, 5 x 8 inch base	1
Water, deionized	300 mL

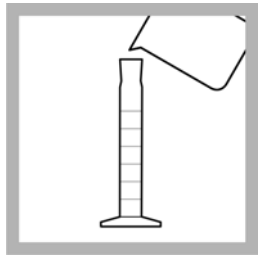
See *Consumables and replacement items* for reorder information.

4-Aminoantipyrine Method

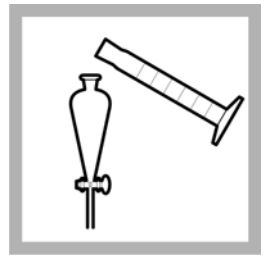


1. Select the test.

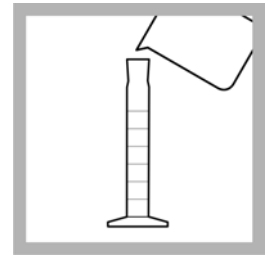
Insert an adapter if required (see *Instrument-specific information*).



2. Measure 300 mL of deionized water in a 500-mL graduated cylinder.



3. Blank Preparation: Pour the measured deionized water into a 500-mL separatory funnel.

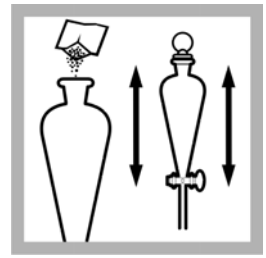


4. Measure 300 mL of sample in a 500-mL graduated cylinder.

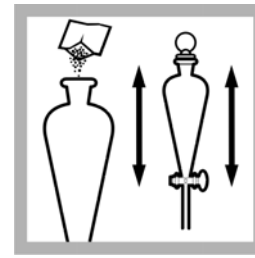
4-Aminoantipyrine Method (continued)

5. Prepared Sample: Pour the measured sample into another 500-mL separatory funnel.

6. Add 5 mL of Hardness Buffer to each separatory funnel. Stopper and shake to mix.

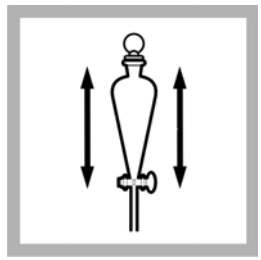


7. Add the contents of one Phenol Reagent Powder Pillow to each separatory funnel. Stopper and shake to dissolve.

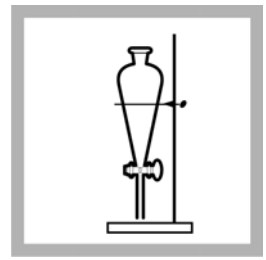


8. Add the contents of one Phenol 2 Reagent Powder Pillow to each separatory funnel. Stopper and shake to dissolve.

9. Add 30 mL of chloroform to each separatory funnel. Stopper each funnel.



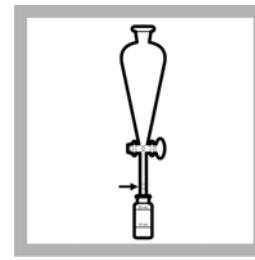
10. Invert each funnel and temporarily vent. Shake each funnel briefly and vent. Then vigorously shake each funnel for a total of 30 seconds (venting if necessary).



11. Remove the stoppers. Allow both funnels to stand until the chloroform settles to the bottom of the funnel.

The chloroform layer will be yellow to amber if phenol is present.

Note: *Make sure that steps 12 through 16 are performed quickly because chloroform will evaporate, causing high readings.*



12. Insert a large, pea-sized cotton plug into the delivery tube of each funnel.

Filtering the chloroform layer through the cotton removes suspended water or particles. The volume of chloroform extract will be about 25 mL.

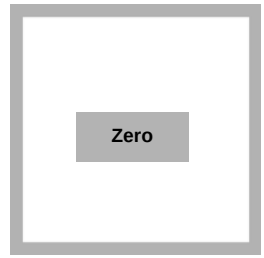
4-Aminoantipyrine Method (continued)

13. Drain the chloroform layers into separate sample cells (one for the blank and one for each sample).

Stopper the cells.

The water phase contains chloroform, which is hazardous. Dispose properly.

14. Wipe the blank and insert it into the cell holder



15. **ZERO** the instrument.

The display will show:

0.000 mg/L Phenol

16. Wipe the prepared sample and insert it into the cell holder

READ the results in mg/L Phenol.

Interferences

Table 294 Interfering substances

Interfering substance	Interference level
pH	The sample pH must be between 3 and 11.5 for best results.
Oxidizing or reducing agents	May interfere. Distill samples (see procedure below).
Sulfides or suspended matter	**1.** Distillation or the following pretreatment is necessary: **2.** Fill a clean 500-mL graduated cylinder with 350 mL of sample. Pour the sample into a clean 500-mL Erlenmeyer flask. **3.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow[1]. Swirl to mix. **4.** Filter 300 mL of the sample through a folded filter paper[1]. Use this solution in step 4.

[1] See *Distillation reagents and apparatus*.

Sample collection, preservation and storage

Most reliable results are obtained when samples are analyzed within four hours after collection. Use the following storage instructions only if prompt analysis is not possible:

1. Collect 500 mL of sample in clean glass containers and add the contents of two Copper Sulfate Powder Pillows.
2. Adjust the pH to 4 or less with 10% Phosphoric Acid Solution. Store at 4 °C (39 °F) or lower and analyze within 24 hours.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Note: *For greater accuracy, analyze standard solutions when new lots of reagent are first used.*

Required for accuracy check:

- Phenol, ACS
- Deionized water
- 1000-mL Class A volumetric flasks
- 500-mL Class A volumetric flask
- 10 mL Class A volumetric pipet and safety bulb
- TenSette Pipet and tips

1. Prepare a 1000-mg/L phenol stock solution as follows:
   a. Weigh 1.000 g of Phenol ACS.
   b. Transfer the phenol to a 1000-mL volumetric flask.
   c. Dilute to the mark with freshly boiled and cooled deionized water and mix to dissolve.
2. Prepare a 10-mg/L working phenol solution as follows:
   a. Pipet 10.0 mL of the 1000-mg/L stock solution to a 1000-mL volumetric flask.
   b. Dilute to the mark with deionized water.
3. Prepare a 0.200-mg/L standard solution as follows:
   a. Pipet 10.0 mL of the 10-mg/L working solution to a 500-mL volumetric flask.
   b. Dilute to the mark with deionized water.
4. Use this solution in place of the sample. Follow the *4-Aminoantipyrine Method* test procedure.
5. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS> MORE>STANDARD ADJUST**.
6. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Distillation

Sample distillation as described in the following steps will eliminate interferences. The sample pH must be between 3 and 11.5 for the best results. See the *Interfering substances* table for pretreatment guidelines.

1. Set up the Distillation Apparatus* by assembling the general purpose apparatus as shown in the Distillation Apparatus Manual. Use the 500-mL Erlenmeyer flask to collect the distillate. It may be necessary to use a laboratory jack to elevate the flask.
2. Place a stirring bar into the flask.
3. Measure 300 mL of water sample in a clean 500-mL graduated cylinder. Pour it into the distillation flask.

* See *Distillation reagents and apparatus*.

4. For proof of accuracy, use a 0.200-mg/L phenol standard (see *Accuracy check*) in addition to the sample.
5. Using a serological pipet, add 1 mL of Methyl Orange Indicator to the distillation flask.
6. Turn on the stirrer power switch. Set the stir control to 5.
7. Add 10% Phosphoric Acid Solution drop-wise until the indicator changes from yellow to orange.
8. Add the contents of one Copper Sulfate Powder Pillow and allow to dissolve (omit this step if copper sulfate was used to preserve the sample). Cap the distillation flask.
9. Turn the water on and adjust it so a constant flow is maintained through the condenser. Set the heat control to 10.
10. Collect 275 mL of distillate in the Erlenmeyer flask, then turn the heat off.
11. Fill a 25-mL graduated cylinder to the 25-mL mark with deionized water. Add the water to the distillation flask.
12. Turn the still back on. Heat until another 25-mL of distillate is collected.
13. Using a clean graduated cylinder, re-measure the distillate to make sure that 300 mL has been collected. The distillate is ready for analysis.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
470	0.100 mg/L phenol	0.093–0.107 mg/L phenol	0.002 mg/L phenol

Summary of method

The 4-aminoantipyrine method measures all ortho- and meta-substituted phenols. These phenols react with 4-aminoantipyrine in the presence of potassium ferricyanide to form a colored antipyrine dye. The dye is then extracted from the aqueous phase with chloroform and the color is measured at 460 nm. The sensitivity of the method varies with the type of phenolic compound. Because water samples may contain various types of phenolic compounds, the test results are expressed as the equivalent concentration of phenol.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Phenols Reagent Set (100 Tests), includes:	—	—	2243900
(2) Chloroform, ACS	60 mL	4 L	1445817
(3) Hardness 1 Buffer Solution, pH 10.1	10 mL	500 mL	42449
(2) Phenol 2 Reagent Powder Pillows	2	100/pkg	183699
(2) Phenol Reagent Powder Pillows	2	100/pkg	87299
Water, deionized	300 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers	1	each	96800
Cotton Balls	1	100/pkg	257201
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 500-mL	1	each	50849
Funnel, separatory, 500-mL	2	each	52049
Pipet Bulb, safety	1	each	1465100
Pipet, volumetric, Class A, 5.00-mL	1	each	1451537
Ring, support, 4-inch	2	each	58001
Support for Ring Stand, 5 x 8 inch base	2	each	56300

Distillation reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity, 100–240 VAC	each	2936701
Copper Sulfate Powder Pillows	50/pkg	1481866
Distillation Heater and Support Apparatus, 115 VAC	each	2274400
Distillation Heater and Support Apparatus, 230 VAC	each	2274402
Distillation Apparatus Set, general purpose	each	2265300
Filter Paper, 12.5 cm	100/pkg	189457
Flask, Erlenmeyer, 500 mL	each	50549
Funnel, 65 mm poly	each	108367
Methyl Orange Indicator Solution, 0.5-g/L	100 mL MDB	14832
Phenol, ACS	113 g	75814

Distillation reagents and apparatus (continued)

Description	Unit	Catalog number
Phosphoric Acid Solution, 10%	100 mL MDB	1476932
Sulfide Inhibitor Reagent Powder Pillows	100/pkg	241899
pH Paper, 0–14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, –10 to 225 °C	each	2635700
Pipet, TenSette®, Pipet, 1.0–10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 19700–10	250/pkg	2199725
Pipet Tips, for TenSette Pipet 19700–10	50/pkg	2199796
Weighing Paper, 76 x 76 mm	500/pkg	1473800
Goggles, Safety vented	each	2550700
Gloves, Chemical Resistant, 9–9½ inch[1]	1 pair	2410104
Flask, volumetric class A, 1000 mL	each	1457453
Flask, volumetric class A, 500 mL	each	1457449

[1] other sizes are available

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Edition 7

Phosphonates

DOC316.53.01109

Persulfate UV Oxidation Method[1] **Method 8007**

Multiple Ranges from 0.02 to 125.0 mg/L **Powder Pillows**

Scope and Application: For boiler and cooling water, wastewater and seawater

[1] Adapted from Blystone, P., Larson, P., *A Rapid Method for Analysis of Phosphate Compounds*, International Water Conference, Pittsburgh, PA. (Oct 26-28, 1981)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 295 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Clean glassware with 1:1 Hydrochloric Acid Solution, followed by a distilled water rinse. Do not clean glassware with commercial detergent.
Wear UV safety goggles while the UV lamp is on.
Do not handle the UV lamp surface. Fingerprints will etch the glass. Wipe the lamp with a soft, clean tissue between samples
The digestion in step 7 is normally completed in less than 10 minutes. However, high-organic loaded samples or a weak lamp can cause incomplete phosphate conversion. To check conversion efficiency, perform a longer digestion and make sure the readings do not increase.

Collect the following items:

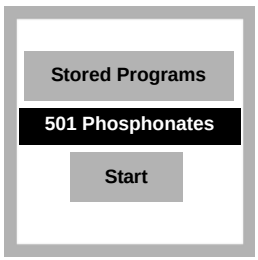
Description	Quantity
Bottle, square, with 25-mL mark	1
Cylinder, mixing, graduated, 50-mL	1
Goggles, UV safety	1
Pipet, serological, 10-mL	1
PhosVer® 3 Phosphate Reagent Powder Pillows	2
Potassium Persulfate Powder Pillow for Phosphonate	1

Collect the following items: (continued)

Description	Quantity
Safety bulb	1
Sample Cells (see *Instrument-specific information*)	2
Water, deionized	varies
UV Lamp with Power Supply	1

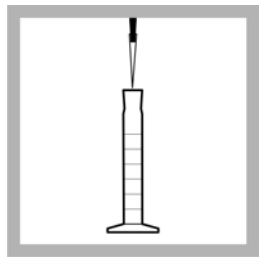
See *Consumables and replacement items* for reorder information.

Persulfate UV Oxidation method for powder pillows

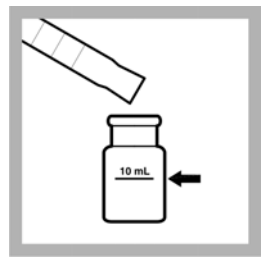


1. Select the test.

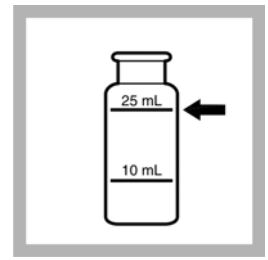
Insert an adapter if required (see *Instrument-specific information*).



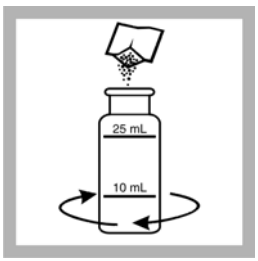
2. Choose the appropriate sample size from the *Expected ranges with multipliers* table. Pipet the chosen volume into a 50-mL graduated cylinder. If necessary, dilute the sample to 50-mL with deionized water and mix well.



3. Blank Preparation: Fill a sample cell to the 10-mL mark with diluted sample from step 2.

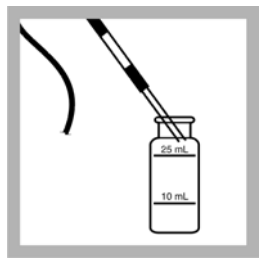


4. Digested Sample: Fill a sample mixing bottle to the 25-mL mark with diluted sample from step 2.



5. Add the contents of one Potassium Persulfate for Phosphonate Powder Pillow to the bottle containing 25 mL of sample.

Swirl to dissolve the powder.



6. Insert the ultraviolet (UV) lamp into the sample bottle.

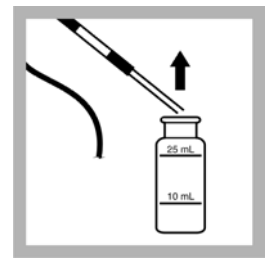
WARNING
Wear UV safety goggles while the lamp is on.

7. Turn on the UV lamp.

Start the instrument timer.

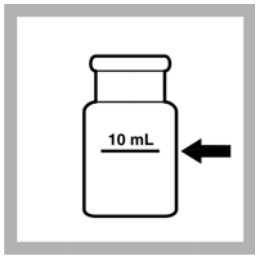
A ten-minute reaction period will begin.

Phosphonates are converted to orthophosphate in this step.



8. When the timer expires, turn off the UV lamp and remove it from the sample.

Persulfate UV Oxidation method for powder pillows



9. Prepared Sample: Fill a second sample cell to the 10-mL mark with the digested sample.

10. Add the contents of one PhosVer 3 Phosphate Reagent Powder Pillow to the **blank and prepared sample**. Immediately swirl vigorously 20–30 seconds to mix. Some powder may not dissolve.

A blue color will develop if phosphate is present. Both sample and blank cells may develop color. The increase in sample color is proportional to the phosphonate concentration.

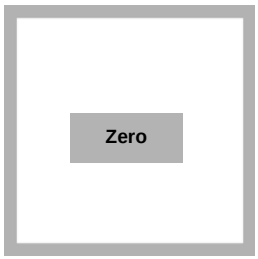
11. Start the instrument timer.

A two-minute reaction period will begin.

If the sample is colder than 15 °C, allow four minutes for color development.

12. When the timer expires, insert the blank into the cell holder.

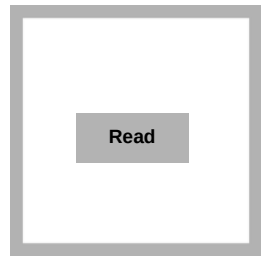
Complete steps 13–16 within three minutes after the timer expires.



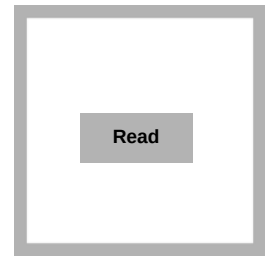
13. ZERO the instrument.

The display will show: 0.00 mg/L PO_4^{3-}

14. Wipe the prepared sample and insert it into the cell holder.



15. READ the results in mg/L PO_4^{3-}.



16. Multiply the value in step 15 by the appropriate multiplier in the *Expected ranges with multipliers* table to obtain the actual phosphonate concentration.

Table 296 Expected ranges with multipliers

Expected range (mg/L phosphonate)	Sample volume (mL)	Multiplier
0 – 2.5	50	0.1
0 – 5	25	0.2

Table 296 Expected ranges with multipliers

Expected range (mg/L phosphonate)	Sample volume (mL)	Multiplier
0 – 12.5	10	0.5
0 – 25	5	1.0
0 – 125	1	5.0

To express results in terms of active phosphonate, multiply the final value in step 16 by the appropriate conversion factor list in the *Conversion factors by phosphonate type* table.

Table 297 Conversion factors by phosphonate type

Phosphonate type	Conversion factor
PBTC	2.84
NTP	1.050
HEDPA	1.085
EDTMPA	1.148
HMDTMPA	1.295
DETPMPA	1.207
HPA	1.49
active phosphonate (mg/L) = phosphonate concentration from step 16 x conversion factor	

Interferences

Interference levels decrease as the sample size increases. For example, copper does not interfere at or below 100 mg/L for a 5.00 mL sample. If the sample volume is increased to 10 mL, copper will begin to interfere above 50 mg/L.

Table 298 Interfering substances

Interfering substance	Interference level (using 5 mL of sample)
Aluminum	100 mg/L
Arsenate	Interferes at all levels
Benzotriazole	10 mg/L
Bicarbonate	1000 mg/L
Bromide	100 mg/L
Calcium	5000 mg/L
CDTA	100 mg/L
Chloride	5000 mg/L
Chromate	100 mg/L
Copper	100 mg/L
Cyanide	100 mg/L (Increase the UV digestion to 30 minutes.)
Diethanoldithiocarbamate	50 mg/L
EDTA	100 mg/L
Iron	200 mg/L
Nitrate	200 mg/L
NTA	250 mg/L
Orthophosphate	15 mg/L
Phosphites and organophosphorus compounds	Reacts quantitatively. Meta- and polyphosphates do not interfere.
Silica	500 mg/L
Silicate	100 mg/L
Sulfate	2000 mg/L
Sulfide	Interferes at all levels
Sulfite	100 mg/L
Thiourea	10 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

- Collect samples in acid-cleaned (1:1 HCl) plastic or glass bottles that have been rinsed with distilled water. Do not use a commercial detergent.
- If prompt analysis is impossible, preserve the sample by adjusting to pH 2 or less with Sulfuric Acid (about 2 mL per liter).
- Store at 4 °C (39 °F). Preserved samples may be stored up to 24 hours.
- Correct the test result for volume additions.

Accuracy check

Standard solution method
Ideally, prepare a solution containing the exact phosphonate product to be tested. This will check the UV conversion of phosphonate to orthophosphate. Alternatively, a phosphate standard can be used to check the accuracy of the colorimetric part of the method.

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate Standard Solution, 1 mg/L
- Deionized water

Use 10 mL of a Phosphate Standard Solution, 1 mg/L solution in place of the sample beginning at step 9 of the *Persulfate UV Oxidation method for powder pillows* test procedure. Use deionized water for the blank. A multiplier value from the *Expected ranges with multipliers* table is not needed. The expected result is 10.0 mg/L phosphate, due to a factor of 10 in the calibration.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution
501	DR 5000	2.00 mg/L PO_4^{3-}	1.97–2.03 mg/L PO_4^{3-}

Sensitivity

The sensitivity depends on the sample volume. Sensitivity is expressed as PO_4^{3-} in this table. Use the *Conversion factors by phosphonate type* table to express as a specific phosphonate.

Range (mg/L)	Volume (mL)	Change in concentration per 0.010 Abs
0–2.5	50	0.02 mg/L PO_4^{3-}
0–5	25	0.04 mg/L PO_4^{3-}
0–12.5	10	0.10 mg/L PO_4^{3-}
0–25	5	0.20 mg/L PO_4^{3-}
0–125	1	1.00 mg/L PO_4^{3-}

Summary of method

This method is directly applicable to boiler and cooling tower samples. The procedure is based on a UV-catalyzed oxidation of phosphonate to orthophosphate. The orthophosphate reacts with the molybdate in the PhosVer 3 reagent to form a mixed phosphate/molybdate complex. This complex is reduced by the ascorbic acid in the PhosVer 3, yielding a blue color that is proportional to the phosphonate present in the original sample. The orthophosphate present in the original sample is subtracted out by preparing the blank and using it to set zero concentration. Test results are measured at 880 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Phosphonate Reagent Set for 10-mL sample (100 tests), includes:	—	—	2429700
PhosVer® 3 Phosphate Reagent Powder Pillows, 10-mL	2	100/pkg	2106069
Potassium Persulfate Powder Pillow for Phosphonate	1	100/pkg	2084769
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Bottle, square, with 25-mL mark	1	each	1704200
Polypropylene Beaker, 50-mL, low form	1	each	108041
Cylinder, mixing, graduated, 50-mL	1	each	189641
Goggles, UV safety	1	each	2113400
Pipet, serological, graduated, 10-mL	1	each	53238
Safety Bulb	1	each	1465100
UV Lamp with Power Supply, 115 VAC	1	each	2082800
OR			
UV Lamp with Power Supply, 230 VAC	1	each	2082802
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 1-mg/L	500 mL	256949

Optional reagents and apparatus

Description	Unit	Catalog number
Hydrochloric Acid Solution, 1:1	500 mL	88449
Sulfuric Acid, ACS Grade	500 mL	97949
Thermometer, –10 to 225 °C, 405 mm	each	2635700
pH paper, 0–14 pH	100/pkg	2601300
Ampule Breaker	each	2196800
Phosver 3 Phosphate Reagent Powder Pillows, 10 mL	1000/pkg	2106028
UV Lamp, shortwave, pencil type	each	2671000
Power Supply, 115V/60Hz	each	2670700
Power Supply, 220V/50Hz	each	2670702

Optional standards

Description	Unit	Catalog number
Phosphate Standard Solution, 3 mg/L	946 mL	2059716
Phosphate Standard Solution, 10 mg/L	946 mL	1420416
Phosphate Standard Solution, 15 mg/L	100 mL	1424342
Phosphate Standard Solution, 30 mg/L	946 mL	1436716
Phosphate Standard Solution, 50 mg/L, 10 mL ampules	16/pkg	17110
Phosphate Standard Solution, 100 mg/L	100 mL	1436832
Phosphate Standard Solution, 500 mg/L, 10 mL ampules	10/pkg	1424210
Phosphate Standard Solution	100 mL	1424232

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Edition 7

Phosphorus, Acid Hydrolyzable Digestion

DOC316.53.01110

USEPA[1] Acid Digestion Method[2] — Method 8180

Scope and Application: For water, wastewater, and seawater

[1] USEPA Accepted for wastewater analyses

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater* 4500-P B & E

Test preparation

Before starting the test:

Rinse all glassware with 1:1 hydrochloric acid. Rinse again with deionized water.

The results of the reactive phosphorus test after the digestion will include the orthophosphate and the acid-hydrolyzable (condensed) phosphate. The condensed phosphate concentration is determined by subtracting the result of an orthophosphate test from this result. Make sure that both results are in the same units, either mg/L PO_4^{3-} or mg/L P before subtracting. The result from this Acid Hydrolyzable test is subtracted from the result of a total phosphorus test to determine organic phosphorus.

Collect the following items:

Description	Quantity
Sodium Hydroxide Solution, 5.0 N	2 mL
Sulfuric Acid Solution, 5.25 N	2 mL
Water, deionized	varies
Cylinder, graduated, 25-mL	1
Flask, Erlenmeyer, 125-mL	1
Hot Plate	1

See *Consumables and replacement items* for reorder information.

Acid digestion

1. Use a graduated cylinder to measure 25 mL of sample. Pour the sample into a 125-mL Erlenmeyer flask.

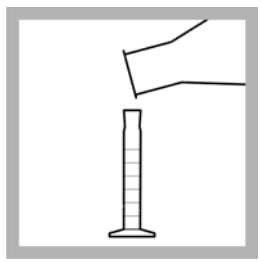
2. Use a 1-mL calibrated dropper to add 2.0 mL of 5.25 N Sulfuric Acid Solution to the flask.

3. Place the flask on a hot plate. Boil gently for 30 minutes. Do not boil dry.

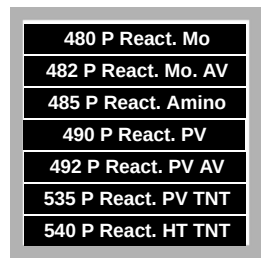
Concentrate the sample to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.

4. Cool the sample to room temperature.

5. Use a 1-mL calibrated dropper to add 2.0 mL of 5.0 N Sodium Hydroxide Solution to the flask. Swirl to mix.



6. Pour the sample into a 25-mL graduated cylinder. Adjust the volume to 25 mL with deionized water rinsings from the flask.



7. Proceed with a reactive phosphorus test of the expected acid hydrolyzable phosphorus concentration range.

Extend the color development time to 10 minutes for the PhosVer 3 method.

Interferences

Table 299 Interfering substances

Interfering substance	Interference level
Alkaline or highly buffered samples	It may be necessary to add additional acid in step 2 to drop the pH of the solution below 1.
Turbidity	Use 50 mL of sample and double the reagent quantities. Use a portion of the digested sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure.

Sample collection, preservation and storage

- Analyze the samples immediately for the most reliable results.
- If prompt analysis is not possible, samples may be preserved up to 28 days. Filter immediately and store at 4 °C (39 °F).

Summary of method

Phosphates present in condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat hydrolyzes the condensed inorganic forms to orthophosphate.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determining the phosphorus content of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is USEPA accepted for NPDES reporting.

Consumables and replacement items

The following reagents and apparatus are required in addition to those required for the active phosphorus test.

Required reagents

Description	Quantity/Test	Unit	Catalog number
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL MDB	245032
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL MDB	244932
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 25-mL	1	each	50840
Flask, Erlenmeyer, 125-mL	1	each	50543
Hot Plate, 7” x 7” Digital, 120 VAC	1	each	2881500
Hot Plate Stirrer, 7” x 7” Digital, 220–240 VAC	1	each	2881602

Required apparatus (field applications)

Description	Unit	Catalog number
Heatab Cookit, with 1 box Heatabs	each	220600
Heatab Replacements	21/pkg	220700

Optional reagents and apparatus

Description	Unit	Catalog number
Sodium, Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
pH paper, 0–14 pH range	100/pkg	2601300
Filter Paper, folded 12.5 cm	100/pkg	69257
Filter Funnel, Analytical, 65 mL	each	108367
Thermometer, Non-Mercury, –10 to 225 °C	each	2635700
Hydrochloric Acid, 6.0 N	500 mL	88449
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

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Edition 7

Phosphorus, Acid Hydrolyzable

DOC316.53.01111

PhosVer™ 3 with Acid Hydrolysis Method **Method 8180**

0.06 to 3.50 mg/L PO_4^{3-} **Test 'N Tube™ Vials**

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 300 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse with deionized water. Do not use detergents that contain phosphate to clean glassware.
Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Refer to the current MSDS for safe handling and disposal instructions.

Collect the following items:

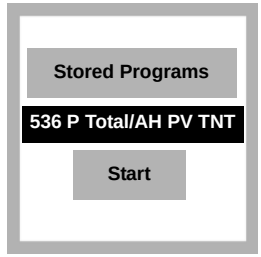
Description	Quantity
Total and Acid Hydrolyzable Phosphorus Reagent Set	1
Deionized water	varies
DRB200 Reactor	1
Funnel, micro	1
Light Shield or Adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 1 to 10 mL, plus tips	
Test Tube Rack	1

See *Consumables and replacement items* for reorder information.

Acid Hydrolysis, TNT method

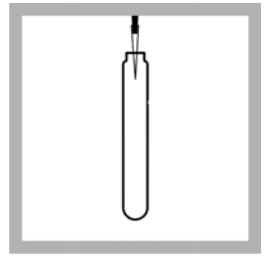
1. Turn on the DRB200 Reactor. Preheat to 150 °C.

See the DRB200 User Manual for selecting pre-programmed temperature applications.



2. Select the test.

Insert an adapter or light shield if required (see *Instrument-specific information*).

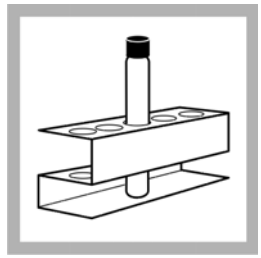


3. Use a TenSette Pipet to add 5 mL of sample to a Total and Acid Hydrolyzable Test Vial. Cap and mix.

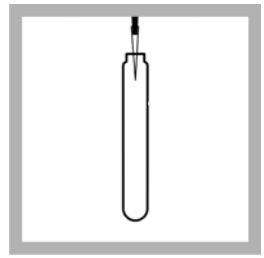
4. Insert the vial into the preheated DRB200 reactor. Close the protective cover.

5. Start the instrument timer.

A 30-minute heating period will begin.



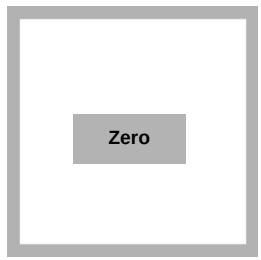
6. After the timer expires, carefully remove the vial from the reactor. Insert it in a test tube rack and cool to room temperature.



7. Using a TenSette Pipet, add 2 mL of 1.00 N sodium hydroxide to the vial. Cap tightly and shake to mix.

8. Clean the outside of the vial with a towel to remove fingerprints or other marks.

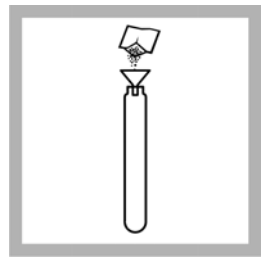
9. Insert the sample vial into the 16-mm round cell holder.



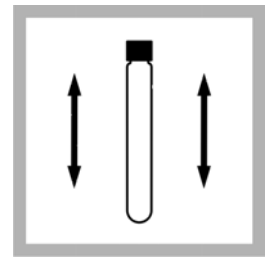
10. **ZERO** the instrument.

The display will show:

0.00 mg/L PO_4^{3-}



11. Using a funnel, add the contents of one PhosVer 3 Powder Pillow to the vial.



12. Immediately cap tightly and shake to mix for 10–15 seconds. The powder will not completely dissolve.

Acid Hydrolysis, TNT method (continued)

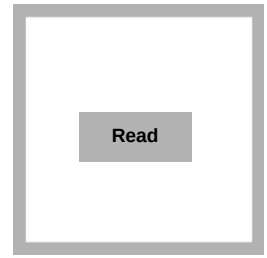
13. Start the instrument timer.

A two-minute reaction period will begin.

Read results within two to eight minutes after adding the PhosVer 3 reagent.

14. Clean the outside of the vial with a towel to remove fingerprints or other marks.

15. Wipe the prepared sample and insert it into the 16-mm round cell holder.



16. **READ** the results in mg/L PO_4^{3-}.

Interferences

Table 301 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	All levels
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Sulfide	Greater than 9 mg/L. Remove sulfide interference as follows: **1.** Measure 25 mL of sample into a 50-mL beaker. **2.** Swirling constantly, add Bromine Water drop-wise until a permanent yellow color appears. **3.** Swirling constantly, add Phenol Solution drop-wise just until the yellow color disappears. Proceed with step 1.
Turbidity	Large amounts may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately for best results. If prompt analysis is not possible, preserve samples by filtering immediately and storing the sample at 4 °C (39 °F) for up to 48 hours.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 2-mL Ampule Standard, 50-mg/L as PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume, and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL, and 0.3 mL of standard to three 25-mL portions of fresh sample.
6. Follow the *Acid Hydrolysis, TNT method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate Standard Solution. 3.0-mg/L

1. Use the Phosphate Standard Solution. 3.0-mg/L solution in place of the sample. Follow the *Acid Hydrolysis, TNT method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the softwarE: **OPTIONS>MORE>STANDARD ADJUST**. .
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—DConcentration per 0.010 DAbs
536	DR 5000	3.00 mg/L PO_4^{3-}	2.93–3.07 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}

Summary of method

Phosphates present in condensed inorganic forms (meta-, pyro-, or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreating the sample with acid and heat hydrolyzes the condensed inorganic forms to orthophosphate.

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total and Acid Hydrolyzable Phosphorus Reagent Set, includes:		50 tests	2742745
PhosVer 3 Phosphate Reagent Powder Pillows	1 pillow	50/pkg	2106046
Potassium Persulfate Powder Pillows	1 pillow	50/pkg	2084766
Sodium Hydroxide, 1.54 N	varies	100 mL	2743042
Sodium Hydroxide Standard Solution, 1.00 N	2 mL	100 mL	104542
Total and Acid Hydrolyzable Test Vials[1]	1 vial	50/pkg	—
Water, deionized	varies	100 mL	27242

[1] Not sold separately.

Required apparatus

Description	Quantity	Unit	Catalog number
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Pipet, volumetric, Class A, 2.00-mL	1	each	1451536
Pipet, volumetric, Class A, 5.00-mL	1	each	1451537
Pipet Filler, safety bulb	1	each	1465100
Pipet, TenSette®, 1 to 10 mL	1	each	1970010
Pipet Tips for TenSette Pipet 19700-10	1	250/pkg	2199725
Test Tube Rack	1	each	1864100

Recommended standards

Description	Unit	Catalog number
Drinking Water Standard, Mixed Parameter, Inorganic for F^-, NO_3, PO_4, SO_4	500 mL	2833049
Phosphate Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as PO_4^{3-}	16/pkg	17110
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL	256949
Phosphate Standard Solution, 3 mg/L as PO_4^{3-}	946 mL	2059716
Wastewater Standard, Effluent Inorganics, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water, 30 g/L	29 mL	221120
Cylinder, mixing, 25 mL	each	189640
Hydrochloric Acid Solution, 6.0 N, 1:1	500 mL	88449
Phenol Solution, 30 g/L	29 mL	211220
pH paper, 0–14 pH range	100/pkg	2601300
Filter Paper, folded 12.5 cm	100/pkg	69257
Filter Funnel, Analytical, 65 mL	each	108367
Thermometer, Non-Mercury, –10 to 225° C	each	2635700
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Pipet Tips for TenSette Pipet 19700–10	50/pkg	2199796
Beaker, 50 mL, Glass	each	50041H

Optional standards

Description	Unit	Catalog number
Voluette Ampule breaker 10 mL	each	2196800
Phosphate, Standard Solution, 3 mg/L	946 mL	2059716
Phosphate, Standard Solution, 10 mg/L	946 mL	1420416
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate, Standard Solution, 30 mg/L	946 mL	1436716
Phosphate, Standard Solution, 50 mg/L, 10 mL ampules	16/pkg	17110
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 500 mg/L, 10 mL ampules	10/pkg	1424210
Phosphate, Standard Solution	100 mL	1424232

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Edition 7

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01115

Molybdovanadate Method[1] **Method 8114**

0.3 to 45.0 mg/L PO_4^{3-} **Reagent Solution or AccuVac® Ampuls**

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 302 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

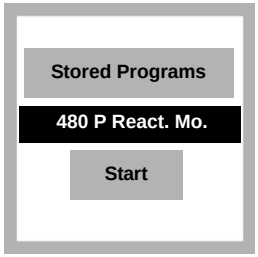
Before starting the test:

For best results, sample temperature should be 20–25 °C (68–77 °F).
After adding reagent, a yellow color will form if phosphate is present. The blank will be slightly yellow because of the reagent.

Description	Quantity
Liquid Reagent Test:	
Molybdovanadate Reagent	1.0 mL
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
Molybdovanadate Reagent AccuVac® Ampuls	2
Beaker, 50-mL	2
Stopper for 18 mm Tube	2

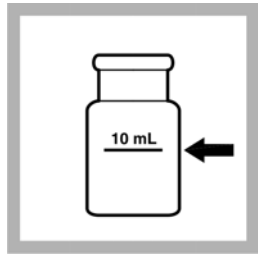
See *Consumables and replacement items* for reorder information.

Molybdovanadate reagent solution

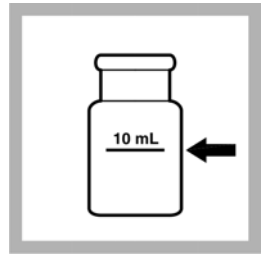


1. Select the test.

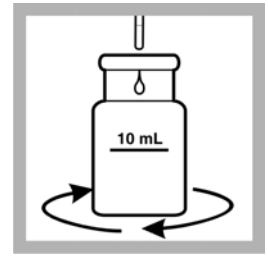
Insert an adapter if required (see *Instrument-specific information*).



2. Blank Preparation: Fill a sample cell with 10 mL of deionized water.



3. Prepared Sample: Fill a second sample cell with 10 mL of sample.



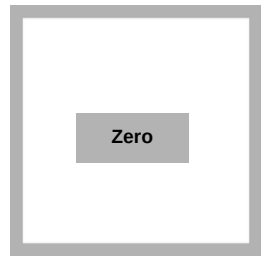
4. Add 0.5 mL of Molybdovanadate Reagent to each sample cell. Swirl to mix.

5. Start the instrument timer.

A 7-minute reaction period will begin.

If the sample concentration is greater than 30 mg/L PO_4^{3-}, read at exactly seven minutes or make a 1:1 dilution of the sample and repeat the test

6. When the timer expires, wipe the blank and insert it into the cell holder.



7. ZERO the instrument.

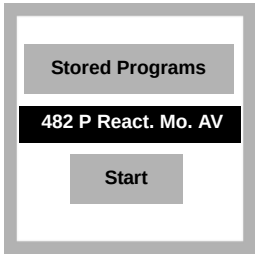
The display will show:

0.0 mg/L PO_4^{3-}

8. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L PO_4^{3-}.

Molybdovanadate for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Prepared Sample: Collect 40 mL of sample in one 50-mL beaker. Fill a Molybdovanadate Reagent AccuVac Ampul with sample.

3. Blank Preparation: Collect 40 mL of deionized water in another 50-mL beaker.

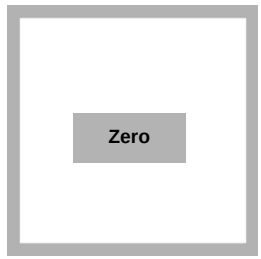
Fill another Ampul with deionized water. Keep the tips immersed while the Ampuls fill completely.

4. Start the instrument timer.

A 7-minute reaction period will begin.

If the sample concentration is greater than 30 mg/L PO_4^{3-}, read at exactly seven minutes or make a 1:1 dilution of the sample and repeat the test.

5. When the timer expires, wipe the blank and insert it into the cell holder.

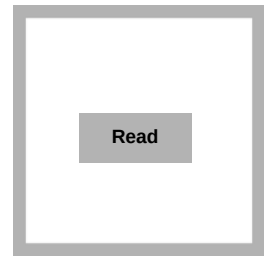


6. ZERO the instrument.

The display will show:

0.0 mg/L PO_4^{3-}

7. Wipe the prepared sample and insert it into the cell holder.



8. READ the results in mg/L PO_4^{3-}.

Interferences

The *Interfering substances* table shows interference levels and types of interference. The *Noninterfering substances at low concentrations (less than 1000 mg/L)* table shows substances that do not interfere in concentrations less than 1000 mg/L.

Table 303 Interfering substances

Interfering substance	Interference level
Arsenate	Only interferes if sample is heated.
Iron, ferrous	Blue color caused by ferrous iron does not interfere if concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Only interferes if sample is heated.

Table 303 Interfering substances (continued)

Interfering substance	Interference level
Sulfide	Causes a negative interference. **1.** Measure 50 mL of sample into an Erlenmeyer flask. **2.** Add Bromine Water[1] drop-wise with constant swirling until a permanent yellow color develops. **3.** Add Phenol Solution[1] drop-wise until the yellow color just disappears. Proceed with step 3 (step 2 if using AccuVac procedure).
pH, extreme or highly buffered samples	May exceed buffering capacity of reagents. May require pretreatment. pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause negative interference.

[1] See *Optional reagents and apparatus*.

Table 304 Noninterfering substances at low concentrations (less than 1000 mg/L)

Pyrophosphate	Tetraborate	Benzoate
Citrate	Lactate	Formate
Oxalate	Tartrate	Salicylate
Al^{3+}	Fe^{3+}	Mg^{2+}
Ca^{2+}	Ba^{2+}	Sr^{2+}
Li^{+}	Na^{+}	K^{+}
NH_4^{+}	Cd^{2+}	Mn^{2+}
NO_3^{-}	NO_2^{-}	SO_4^{2-}
SO_3^{2-}	Pb^{2+}	Hg^{+}
Hg^{2+}	Sn^{2+}	Cu^{2+}
Ni^{2+}	Ag^{+}	U^{4+}
Zr^{4+}	AsO_3^{-}	Br^{-}
CO_3^{2-}	ClO_4^{-}	CN^{-}
IO_3^{-}	SiO_4^{4-}	Selenate

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use a commercial detergent. The phosphate content will contaminate the sample.
- Analyze samples as soon as possible for best results.
- If samples cannot be analyzed promptly, store the sample for up to 48 hours at 4 °C (39 °F) or below.
- Warm stored samples to room temperature before analyzing.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 2-mL Ampule Standard, 500-mg/L PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
6. Pour 10 mL of the spiked samples into three 10 mL sample cells or for AccuVacs, pour 10 mL of the spiked samples into a beaker.
7. Follow the *Molybdovanadate for AccuVac® Ampuls* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate standard solution, 10-mg/L

1. Use the Phosphate standard solution, 10-mg/L, in place of the sample. Follow the *Molybdovanadate for AccuVac® Ampuls* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
480	30.0 mg/L PO_4^{3-}	29.6–30.4 mg/L PO_4^{3-}	0.3 mg/L PO_4^{3-}

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
482	30.0 mg/L PO_4^{3-}	29.7–30.3 mg/L PO_4^{3-}	0.3 mg/L PO_4^{3-}

Summary of method

In the molybdovanadate method, orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, yellow molybdovanadophosphoric acid is formed. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 430 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Molybdovanadate Reagent	1.0 mL	100 mL MDB	2076032
OR			
Molybdovanadate Reagent AccuVac® Ampuls	2	25/pkg	2525025
Water, deionized	25 mL	4 L	27256

Required apparatus (reagent liquid)

Description	Quantity	Unit	Catalog number
Stopper for 18 mm Tube	2	6/pkg	173106

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
AccuVac snapper	1	each	2405200
Sample cell, 10 mL, round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL, round, 25 x 60 mm	1	6/pkg	2427606
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 10-mg/L as PO_4^{3-}	946 mL	1420416
Phosphate Standard Solution, 10-mL PourRite® Ampule, 500 mg/L as PO_4^{3-}	16/pkg	1424210
Wastewater Influent Standard, for mixed parameters NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149
Voluette Ampule breaker, 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water, 30 g/L	29 mL	221120
Cylinder, mixing, 25 mL	each	189640
Hydrochloric Acid, 6.0 N 1:1	500 mL	88449
Phenol Solution, 30 g/L	29 mL	211220
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
pH Paper, 0–14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
AccuVac ampule drainer	each	4103600

[1] Other sizes are available

Optional standards

Description	Unit	Catalog number
Phosphate, Standard Solution, 3 mg/L	946 mL	2059716
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate, Standard Solution, 30 mg/L	946 mL	1436716
Phosphate, Standard Solution, 50 mg/L, 10 mL Voluette Ampules	16/pkg	17110
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01113

Amino Acid Method[1] **Method 8178**

0.23 to 30.00 mg/L PO_4^{3-}

Scope and Application: For water, wastewater and seawater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 305 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

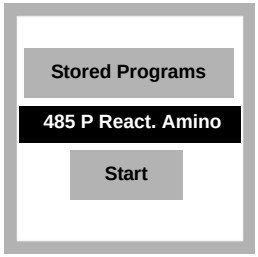
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust
The contents of one Amino Acid Reagent Powder Pillow may be substituted for 1 mL of amino acid reagent solution in step 4.

Collect the following items:

Description	Quantity
Amino Acid Reagent	1 mL
Cylinder, 25-mL, graduated, mixing	1
Molybdate Reagent	1 mL
Sample Cells (see *Instrument-specific information*)	2

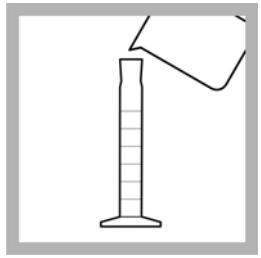
See *Consumables and replacement items* for reorder information.

Amino Acid Method

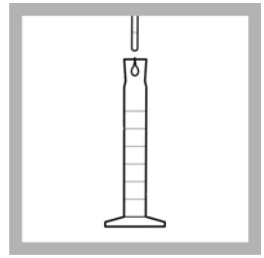


1. Select the test.

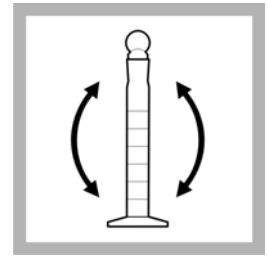
Insert an adapter if required (see *Instrument-specific information*).



2. Fill a 25-mL mixing cylinder with 25 mL of sample.



3. Add 1 mL of Molybdate Reagent using a 1-mL calibrated dropper.

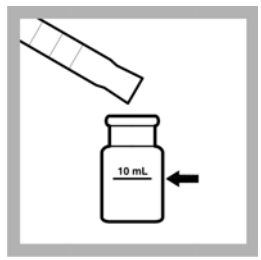


4. **Prepared Sample:** Add 1 mL of Amino Acid Reagent Solution. Stopper and invert several times to mix.

A blue color will form if phosphate is present.

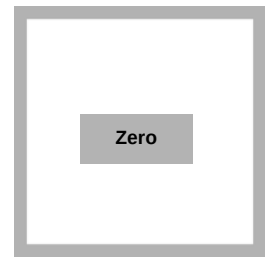
5. Start the instrument timer.

A 10-minute reaction period will begin. Continue with step 6 while the timer is running.



6. **Blank Preparation:** Fill a sample cell with untreated sample.

7. When the timer expires, wipe the blank and insert it into the cell holder.



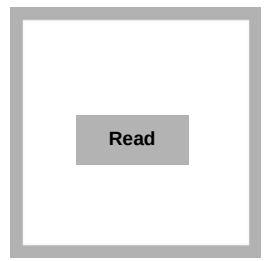
8. **ZERO** the instrument.

The display will show:

0.00 mg/L PO_4^{3-}

9. Fill a second cell with prepared sample.

10. Wipe the prepared sample and insert it into the cell holder.



11. **READ** the results in mg/L PO_4^{3-}.

Interferences

Table 306 Interfering substances

Interfering substance	Interference level
Calcium	Greater than 10,000 mg/L as $CaCO_3$
Chloride	Greater than 150,000 mg/L Cl^-
Colored samples	Add 1 mL of 10 N Sulfuric Acid Standard Solution[1] to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
High salt levels (Na^+)	May cause low results. To eliminate this interference, dilute the sample until two successive dilutions yield about the same result.
Magnesium	Greater than 40,000 mg/L as $CaCO_3$
Nitrite (NO_2^-)	Bleaches the blue color. Remove nitrite interference by adding 0.05 g of sulfamic acid to the sample. Swirl to mix. Continue with step 4.
Phosphates, high levels (PO_4^{3-})	As the concentration of phosphate increases, the color changes from blue to green, then to yellow and finally to brown. The brown color may suggest a concentration as high as 100,000 mg/L PO_4^{3-}. If a color other than blue is formed, dilute the sample and retest.
Sulfide (S^{2-})	Sulfide interferes. For samples with sulfide concentration less than 5 mg/L sulfide interference may be removed by oxidation with Bromine Water as follows: **1.** Measure 50 mL of sample into an Erlenmeyer flask. **2.** Add Bromine Water[1] drop-wise with constant swirling until permanent yellow color develops. **3.** Add Phenol Solution[1] drop-wise until the yellow color just disappears. Use this solution in steps 2 and 6.
Temperature	For best results, sample temperature should be 21 ±3 °C (70 ±5 °F).
Turbidity	May give inconsistent results for two reasons. Some suspended particles may dissolve because of the acid used in the test. Also, desorption of orthophosphate from particles may occur. For highly turbid samples, add 1 mL of 10 N Sulfuric Acid Standard Solution[1] to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water.
- Do not use a commercial phosphate-based detergent for cleaning glassware. The phosphate content will contaminate the sample.
- Analyze samples immediately for best results.
- If prompt analysis is not possible, preserve samples by filtering immediately and store at 4 °C (39 °F) for up to 48 hours.
- The sample should have a neutral pH (6–8) and be at room temperature before analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 2-mL Ampule Standard, 500-mg/L PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
6. Follow the *Amino Acid Method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 10-mg/L Phosphate Standard Solution

1. Use the 10-mg/L Phosphate Standard Solution in place of the sample. Follow the *Amino Acid Method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
485	DR 5000	10.00 mg/L PO_4^{3-}	9.86–10.14 mg/L PO_4^{3-}	0.20 mg/LPO_4^{3-}

Summary of method

In a highly acidic solution, ammonium molybdate reacts with orthophosphate to form molybdophosphoric acid. This complex is then reduced by the amino acid reagent to yield an intensely colored molybdenum blue compound. Test results are measured at 530 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
High Range Reactive Phosphorus Reagent Set, includes:	—	100 tests	2244100
Amino Acid Reagent	1 mL	100 mL MDB	193432
Molybdate Reagent	1 mL	100 mL MDB	223632

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Cylinder, 25-mL, graduated, mixing	1	each	189640

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 10-mg/L	946 mL	1420416
Phosphate Standard Solution, 2-mL PourRite® Ampule, 500-mg/L PO_4^{3-}	16/pkg	1424220
Wastewater Effluent Standard, for mixed parameters NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Wastewater Influent Standard for mixed parameters NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149
Water, deionized	4 liters	27256
PourRite Ampule breaker, 2 mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Amino Acid Reagent Powder Pillow	100/pkg	80499
Bromine Water, 30 g/L	29 mL	221120
Flask, Erlenmeyer, 125 mL	each	50543
Hydrochloric Acid Solution, 1:1, 6.0 N	500 mL	88449
Phenol Solution, 30g/L	29 mL	211220
Sulfamic Acid	454 g	234401
Sulfuric Acid Standard Solution, 10 N	1000 mL	93153
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
pH Paper, 0 - 14 pH range	100/pkg	2601300
Filter Paper, folded 12.5 cm	100/pkg	69257
Filter Funnel, Analytical, 65 mL	each	108367
Thermometer, Non-Mercury, -10 to 225°C	each	2635700
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

[1] Other sizes are available

Optional Standards

Description	Unit	Catalog number
Voluette Ampule breaker 10 mL	each	2196800
Phosphate, Standard Solution, 3 mg/L	946 mL	2059716
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate, Standard Solution, 30 mg/L	946 mL	1436716
Phosphate, Standard Solution, 50 mg/L, 10 mL Voluette Ampules	16/pkg	17110
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate, Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Phosphorus, Reactive

DOC316.53.01114

Molybdovanadate Rapid Liquid Method[1] **Method 8114**

HR (0.3 to 45.0 mg/L PO_4^{3-}) **Pour-Thru Cell**

Scope and Application: For treated and natural waters

[1] Adapted from Standard Methods for the Examination of Water and Wastewater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 307 Instrument-specific information

Instrument	Pour-Thru Kit	Pour-Thru Cell Orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

See the User Manual for Pour-Thru Module installation instructions.
Clean the Pour-Thru cell and all labware as specified in *Analysis labware treatment*.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
Make sure the PourThru cell is completely seated in the sample cell compartment.
Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Refer to the current MSDS for safe handling and disposal information.

Description	Quantity
Molybdovanadate Reagent	2 mL
Water, deionized	25 mL
Cylinder, graduated, 25-mL, poly	1
Dispenser, fixed volume, 1.0-mL, w/bottle	1
Flask, Erlenmeyer, 125-mL, PMP w/cap	2
Instrument-specific information	1

Description	Quantity

See *Consumables and replacement items* for reorder information.

Molybdovanadate Rapid Liquid method

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. I

3. Rinse a clean plastic 125-mL Erlenmeyer flask and a 25-mL graduated cylinder with deionized water.

4. Blank Preparation: Measure 25 mL of deionized water in the graduated cylinder. Pour the water into the flask.

5. Rinse another clean plastic 125-mL Erlenmeyer flask with deionized water.

6. Prepared Sample: Measure 25 mL of sample in the graduated cylinder. Pour the water into the flask.

7. Add 1.0 mL of Molybdovanadate Reagent to each flask using a Repipet Jr. Dispenser. Swirl to mix.

A yellow color will develop in the sample if phosphate is present. A small amount of yellow may be present in the blank due to the reagent.

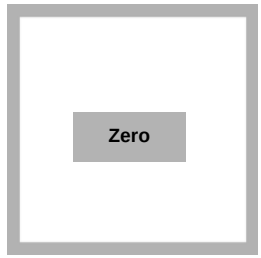
8. Start the instrument timer.

A 7-minute reaction period will begin.

If the sample concentration is greater than 30 mg/L PO_4^{3-}, read at exactly seven minutes or make a 1:1 dilution of the sample and begin the test again.

Molybdovanadate Rapid Liquid method (continued)

9. When the timer expires, pour the blank from the flask into the Pour-Thru Cell.

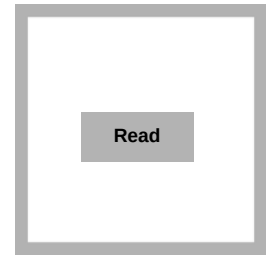


10. **ZERO** the instrument.

The display will show:

0.0 mg/L PO_4^{3-}

11. Pour the prepared sample from the flask into the Pour-Thru Cell.



12. **READ** the results in mg/L PO_4^{3-}.

Flush the Pour-Thru Cell with 50 mL of deionized water.

Interferences

See the *Interfering substances* table for a list of substances, interference levels and type of interference. See the *Noninterfering substances at low concentrations (less than 1000 mg/L)* table for a list of substances that do not interfere in concentrations less than 1000 mg/L.

Table 308 Interfering substances

Interfering substance	Interference level
Arsenate	Negative interference. Positive interference if sample is heated.
Bismuth	Negative interference.
Fluoride	Negative interference.
Iron, Ferrous	Blue color is caused by ferrous iron but this does not affect results if the ferrous iron concentration is less than 100 mg/L.
Molybdate	Negative interference.
Silica	Positive interference if sample is heated.
Sulfide	Negative interference. Sulfide interference may be removed by oxidation with Bromine Water as follows: **1.** Measure 25 mL of sample into a flask. **2.** Add Bromine Water[1] drop-wise with constant swirling until permanent yellow color develops. **3.** Add Phenol Solution[1] drop-wise until the yellow color just disappears. Proceed with step 7.
Thiocyanate	Negative interference.
Thiosulfate	Negative interference.
Thorium	Negative interference.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

[1] See *Optional reagents and apparatus*.

Table 309 Noninterfering substances at low concentrations (less than 1000 mg/L)

Pyrophosphate	Tetraborate	Benzoate
Citrate	Lactate	Formate
Oxalate	Tartrate	Salicylate
Al^{3+}	Selenate	Mg^{2+}
Ca^{2+}	Ba^{2+}	Sr^{2+}
Li^{+}	Na^{+}	K^{+}
NH_4^{+}	Cd^{2+}	Mn^{2+}
NO_3^{-}	NO_2^{-}	SO_4^{2-}
SO_3^{2-}	Pb^{2+}	Hg^{+}
Hg^{2+}	Sn^{2+}	Cu^{2+}
Ni^{2+}	Ag^{+}	U
Zr^{4+}	AsO_3^{-}	Br^{-}
CO_3^{2-}	ClO_4^{-}	CN^{-}
IO_3^{-}	Fe^{3+}	SiO_4^{4-}

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use detergents that contain phosphate for cleaning labware.
- Analyze samples immediately for best results.
- If prompt analysis is not possible, preserve samples by filtering immediately and storing the sample at 4 °C (39 °F) or below for up to 48 hours.
- Warm stored samples to room temperature before analyzing.

Analysis labware treatment

1. Clean containers by normal means (do not use detergents containing phosphorus), then rinse with deionized water.
2. Soak for several minutes in a 1:25 dilution of Molybdovanadate Reagent in deionized water.
3. Rinse well with deionized water. Dedicate these containers for HR PO_4^{3-} analysis.
4. Fill the Pour-Thru Cell with this same mixture of Molybdovanadate reagent and deionized water and let stand for several minutes.
5. Rinse the Pour-Thru Cell with 50 mL of deionized water.

* See *Optional reagents and apparatus*.

How to clean the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. To clean the Pour-Thru Cell:

1. Remove the color by rinsing with a 1:5 dilution of ammonium hydroxide*
2. Follow with several deionized water rinses.
3. Invert a beaker over the glass funnel of the Pour-Thru Cell when not in use.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate Voluette® Ampule Standard Solution, 500-mg/L as PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL mixing cylinders and fill to 25 mL with the fresh sample. Mix thoroughly.
6. Follow the *Molybdovanadate Rapid Liquid method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

* *Optional reagents and apparatus*.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 10.0-mg/L Phosphate Standard

1. Use the 10.0-mg/L Phosphate Standard solution in place of the sample. Follow the *Molybdovanadate Rapid Liquid method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
489	10.0 mg/L PO_4^{3-}	9.9–10.1 mg/L PO_4^{3-}	0.34 mg/L PO_4^{3-}

Summary of method

In the molybdovanadate method, orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 430 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Molybdovanadate Reagent	2 mL	500 mL	2076049
Water, deionized	25 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 25-mL, poly	1	each	108140
Dispenser, adjustable	1	each	2563137
Flask, Erlenmeyer, 125-mL, PMP w/cap	2	each	2089843

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 10-mg/L as PO_4^{3-}	946 mL	1420416
Phosphate Standard Solution, Voluette® ampule, 10-mL, 500-mg/L as PO_4^{3-}	16/pkg	1424210
Wastewater Influent Standard for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, ACS	500 mL	10649
Bromine Water, 30 g/L	29 mL	221120
Cylinder, graduated mixing, 25 mL	each	189640
Hydrochloric Acid Solution, 6.0 N, 1:1	500 mL	88449
Phenol Solution, 30 g/L	29 mL	211220
Pipet, TenSette®, Pipet, 0.1 - 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Filter Paper, folded 12.5 cm	100/pkg	69257
Filter Funnel, Analytical, 65 mL	each	108367
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

[1] Other sizes are available

Optional standards

Description	Unit	Catalog number
Voluette Ampule breaker, 10 mL	each	2196800
Phosphate, Standard Solution, 3 mg/L	946 mL	2059716
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate, Standard Solution, 30 mg/L	946 mL	1436716
Phosphate, Standard Solution, 50 mg/L, 10 mL Voluette Ampules	16/pkg	17110
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Phosphorus, Reactive

DOC316.53.01117

Ascorbic Acid Rapid Liquid Method[1] **Method 10055**

LR (19 to 3000 µg/L PO_4^{3-}) **Pour-Thru Cell**

Scope and Application: For treated and natural waters

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 310 Instrument-specific information

Instrument	Pour-Thru Kit	Pour-Thru Cell Orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

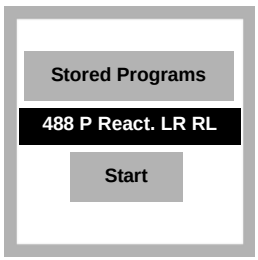
See the user manual for Pour-Thru Module installation instructions.
Clean the Pour-Thru cell and all labware as specified in *Analysis labware treatment*.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
See *Reagent preparation* for preparing the Ascorbic Acid reagent.
Reaction time depends on sample temperature. For most accurate results, samples should be at room temperature (about 20 °C).
Obtain a reagent blank for each lot of reagent when the normal sample phosphate concentration is less than 750 µg/L. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results.
Make sure the PourThru cell is completely seated in the sample cell compartment.

Collect the following items:

Description	Quantity
Ascorbic Acid Reagent Dilution Solution	1 mL
Ascorbic Acid Reagent Powder	varies
Cylinder, graduated, 25 mL, poly	2
Dispenser, bottle top	2
Flask, Erlenmeyer, 125-mL, PMP w/cap	2
Molybdate Reagent Solution	2 mL
Pour-Thru Cell Assembly	1
Water, deionized	varies

See *Consumables and replacement items* for reorder information.

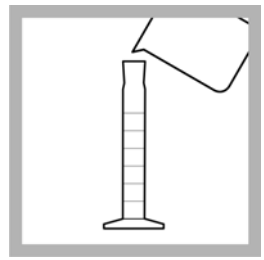
Ascorbic acid method, rapid liquid



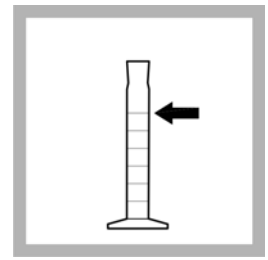
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. Rinse two clean Erlenmeyer flasks three times with the sample.



3. Rinse a clean 25-mL plastic graduated cylinder three times with the sample.



4. Fill the rinsed cylinder to the 25-mL mark with sample.

5. Pour the contents of the 25-mL cylinder into one of the flasks.

6. Measure a second 25-mL portion of sample into the graduated cylinder and pour the contents into the second flask.

7. Add 1.0 mL of Molybdate reagent to each flask using a bottle-top dispenser. Swirl to mix.

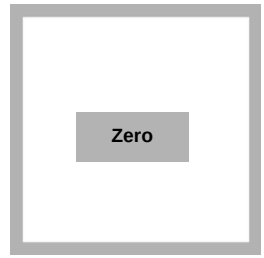
8. Prepared Sample: Add 1.0 mL of prepared Ascorbic Acid reagent to one of the flasks with a bottle-top dispenser. Swirl to mix. The remaining flask will be the blank.

Ascorbic acid method, rapid liquid (continued)

9. Start the instrument timer.

A five-minute reaction period will begin.

10. When the timer expires, pour the contents of the flask that contains the blank into the Pour-Thru Cell.



11. After the flow stops, **ZERO** the instrument.

The display will show:

0 µg/L PO_4^{3-}

12. Pour the prepared sample into the Pour-Thru Cell.

13. **READ** the results in µg/L PO_4^{3-}.

14. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Table 311 Interfering substances

Interfering substance	Interference level
Aluminum	200 mg/L
Arsenate	Interferes
Chromium	100 mg/L
Copper	10 mg/L
Hydrogen sulfide	Interferes
Iron	100 mg/L
Nickel	300 mg/L
Silica	50 mg/L
Silicate	10 mg/L
Turbidity	Samples with large amounts of turbidity may give inconsistent results because the acid present in the reagents may dissolve some of the suspended particles and variable desorption of orthophosphate from the particles may occur.
Zinc	80 mg/L

Table 311 Interfering substances (continued)

Interfering substance	Interference level
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Analysis labware treatment

All labware used in this test must be thoroughly cleaned to remove all traces of phosphate.

1. Clean containers with a non-phosphate detergent followed by a rinse with deionized water.
2. Fill and soak for 10 minutes with a 1:25 dilution of Molybdate Reagent in deionized water.
3. Rinse well with deionized water. Keep containers tightly closed when not in use.
4. Treat the Pour-Thru Cell with this same mixture of molybdate and water followed by thorough rinsing with deionized water.

Dedicate these containers for low-level phosphate analysis. If these containers are rinsed and capped after use, only occasional pre-treatment is necessary.

How to clean the Pour-Thru cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. To remove color:

1. Rinse the Pour-Thru Cell with a 1:5 dilution of Ammonium Hydroxide.
2. Follow the Ammonium Hydroxide rinse with deionized water rinses.
3. Invert a beaker over the glass funnel of the cell when the Pour-Thru Cell is not in use.

Reagent preparation

The Ascorbic Acid reagent must be prepared before use.

1. Using a powder funnel, add the contents of one 48 g bottle of Ascorbic Acid Reagent Powder* to one 450 mL bottle of Ascorbic Acid Reagent Dilution Solution.
2. Invert several times and swirl until the powder is completely dissolved.
3. Attach dispensers to the top of this bottle and the Molybdate Reagent bottle. This solution may develop a yellow color with time but will still give accurate results for up to one month after mixing if stored at 20–25 °C.
4. Record the date of preparation on the bottle and discard any remaining solution after one month.

Do not add fresh reagent to previously mixed reagent. Use of this reagent after one month may result in high reagent blanks and low values at high concentrations.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use detergents that contain phosphate for cleaning labware.
- Analyze samples immediately for best results.
- If prompt analysis is not possible, preserve samples by filtering immediately and storing at 4 °C (39 °F) for up to 48 hours.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate Standard Solution Ampule, 50-mg/L (50,000 µg/L) as PO_4^{3-} (for concentrations greater than 1000 µg/L)
- Phosphate Standard Solution Ampule, 15-mg/L (15,000 µg/L) as PO_4^{3-} (for concentrations less than 1000 µg/L)
- Ampule breaker
- Flask, Erlenmeyer, 125-mL, PMP w/cap
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule needed for the expected concentration.
5. Measure three 25 mL portions of fresh samples into three flasks.
6. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to the three 25-mL portions of fresh sample.
7. Follow the *Ascorbic acid method, rapid liquid* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 1000-µg/L (1.000-mg/L) phosphate standard solution

1. Use the 1000-µg/L (1.000-mg/L) phosphate standard solution in place of the sample. Follow the *Ascorbic acid method, rapid liquid* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
488	DR 5000	1000 µg/L PO_4^{3-}	970–1030 µg/L PO_4^{3-}	21 µg/L PO_4^{3-}

Summary of method

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Reactive phosphorus includes existing orthophosphate in the sample plus a small fraction of condensed phosphate that may be hydrolyzed to orthophosphate during the test. Test results are measured at 880 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Rapid Liquid Low Range Phosphorus Reagent Set, includes:			2678600
Ascorbic Acid Reagent Dilution Solution	1 mL	450 mL	2599949
Ascorbic Acid Reagent Powder	varies	48 g	2651255
Molybdate Reagent Solution	2 mL	500 mL	2599849
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 25-mL, poly	1	each	108140
Adjustable Digital Dispenser	2	each	2563137
Flask, Erlenmeyer, 125-mL, PMP w/cap	2	each	2089843
Powder funnel, 65 mm top ID	1	each	2264467

Recommended standards

Description	Unit	Catalog number
Drinking Water Standard, Mixed Inorganics for NO_3, PO_4, SO_4	500 mL	2833049
Phosphate Standard Solution, 1.00-mg/L as PO_4^{3-}	500 mL	256949
Phosphate Standard Solution, 3-mg/L as PO_4^{3-}	946 mL	2059716
Phosphate Standard Solution, Voluette® ampule, 10-mL, 50-mg/L PO_4^{3-}	16/pkg	17110
Phosphate Standard Solution, 15-mg/L PO_4^{3-}	100 mL	1424342
Wastewater Effluent Standard for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Voluette Ampule breaker, 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, ACS	500 mL	10649
Cylinder, mixing, 25 mL	each	189640
Hydrochloric Acid Solution,6.0 N, 1:1	500 mL	88449
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Filter Paper, fold 12.5 cm	100/pkg	69257
Filter Funnel, Analytical, 65 mL	each	108367
Pipet, TenSette®, Pipet, 0.1 - 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628

[1] Other sizes are available

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Edition 7

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01119

USEPA[1] PhosVer 3 (Ascorbic Acid) Method[2] **Method 8048**

0.02 to 2.50 mg/L PO_4^{3-} **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted for reporting for wastewater analyses. Procedure is equivalent to USEPA and Standard Method 4500-P-E for wastewater.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 312 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

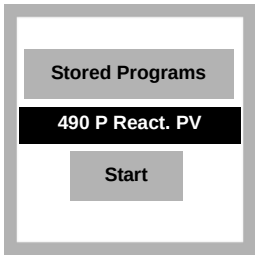
Description	Quantity
Powder Pillow Test:	
PhosVer® 3 Phosphate Reagent powder pillow	1
Sample Cells, 1-inch, 10-mL	2
Stopper for 18 mm Tube	1
AccuVac Test:	
PhosVer® 3 Phosphate Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample Cell, 10-mL round	1

Collect the following items:

Description	Quantity
Stopper for 18-mm Tube (supplied with PhosVer AccuVacs)	1

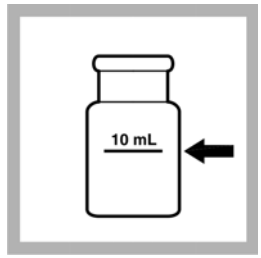
See *Consumables and replacement items* for reorder information.

PhosVer 3 (Ascorbic Acid) method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

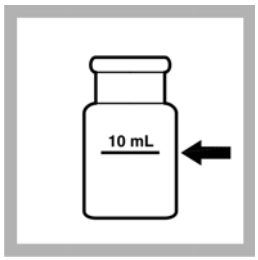


2. Fill a sample cell with 10-mL of sample.

3. Prepared Sample: Add the contents of one PhosVer 3 phosphate Powder Pillow to the cell. Immediately stopper and shake vigorously for 30 seconds.

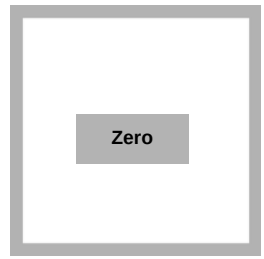
4. Start the instrument timer.

A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.



5. Blank Preparation: Fill a second sample cell with 10 mL of sample.

6. When the timer expires, wipe the blank and insert it into the cell holder.



7. ZERO the instrument.

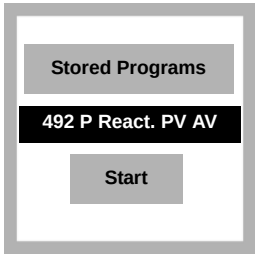
The display will show:

0.00 mg/L PO_4^{3-}

8. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L PO_4^{3-}.

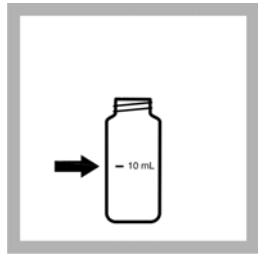
PhosVer 3 (Ascorbic Acid) method for AccuVac® Ampuls



1. Select the test.

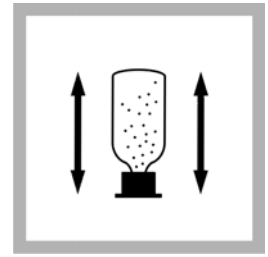
Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a sample cell with 10-mL of sample.

3. Prepared Sample: Fill a PhosVer 3 Phosphate AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.



4. Secure an Ampul cap over the tip of the Ampul. Shake the Ampul for approximately 30 seconds.

Accuracy is unaffected by undissolved powder.

5. Start the instrument timer.

A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.

6. When the timer expires, wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show:

0.00 mg/L PO_4^{3-}

7. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L PO_4^{3-}.

Interferences

Table 313 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level.
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Hydrogen Sulfide	Interferes at any level
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. pH 2–10 is recommended.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Turbidity or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment[1] Powder Pillow to 25 mL of sample. Mix well. Use this solution to zero the instrument.
Zinc	Greater than 80 mg/L

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.
- For best results, analyze samples immediately.
- If prompt analysis is not possible, preserve samples by filtering immediately and storing at 4 °C (39 °F) for up to 48 hours.
- Return the sample to room temperature before analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 10-mL Ampule Standard, 50-mg/L PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **OPTIONS>MORE>STANDARD ADDITIONS** from the instrument menu.

* See *Optional reagents and apparatus*.

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Prepare a 0.1-mL sample spike by adding 0.1 mL of standard to the unspiked sample. Press the timer icon. After the timer expires, read the result.
6. Prepare a 0.2-mL sample spike by adding 0.1 mL of standard to the 0.1-mL sample spike. Press the timer icon. After the timer expires, read the result.
7. Prepare a 0.3-mL sample spike by adding 0.1 mL of standard to the 0.2-mL sample spike. Press the timer icon. After the timer expires, read the result. Each addition should reflect approximately 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)
Required for accuracy check:

- Mixing cylinders (3)

1. Fill three mixing cylinders each with 50-mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of standard.
2. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
3. Analyze each standard addition sample as described in the *PhosVer 3 (Ascorbic Acid) method for AccuVac® Ampuls*.
4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate standard solution, 50 mg/L
- Deionized water
- 100-mL Class A volumetric flask
- 4 mL Class A volumetric pipet and pipet bulb

1. Prepare a 2.00 mg/L phosphate standard solution as follows:
   a. Pipet 4.00 mL of Phosphate Standard, 50-mg/L, into a 100-mL volumetric flask.
   b. Dilute to volume with demineralized water. Mix well. Prepare this solution daily.

Note: *Alternately, use one of the mixed parameter standards listed in Recommended standards. These contain 2.0 mg/L phosphate.*

2. Use this solution in place of the sample. Follow the *PhosVer 3 (Ascorbic Acid) method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
490	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
492	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}

Summary of method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
PhosVer® 3 Phosphate Reagent Powder Pillows, 10-mL	1	100/pkg	2106069
OR			
PhosVer® 3 Phosphate Reagent AccuVac® Ampuls	1	25/pkg	2508025

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
Stopper for 18 mm Tube	1	6/pkg	173106

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Stopper for 18 mm Tube	1	6/pkg	173106
Sample cell, 10 mL round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	each	2122800
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 10-mL Voluette® Ampul, 50-mg/L as PO_4	16/pkg	17110
Phosphate Standard Solution, 50-mg/L as PO_4	500 mL	17149
Phosphate Standard Solution, 1-mg/L as PO_4	500 mL	256949
Standard, Drinking Water, Mixed Parameter, Inorganic: F, NO_3, PO_4, SO_4	500 mL	2833049
Wastewater Effluent Standard, for mixed parameters: NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Hydrochloric Acid Solution, 6.0N, 1:1	500 mL	88449
Mixing Cylinder, 50 mL	each	189641
Phosphate Treatment Powder Pillow	100/pkg	1450199
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Pipet, TenSette, Pipet, 1.0 - 10.0 mL	each	1970010

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet Tips, for TenSette Pipet 1970010[1]	50/pkg	2199796
Pipet Tips, for TenSette Pipet 1970010[1]	250/pkg	2199725
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
pH Paper, 0–14 pH range	100/pkg	2601300
AccuVac snapper	each	2405200
AccuVac ampule blanks	25/pkg	2677925
Flask, volumetric, 100 mL	each	1457442
Pipet, volumetric, Class A, 4 mL	each	1451504
AccuVac ampule drainer	each	4103600

[1] Other sizes are available

Optional standards

Description	Unit	Catalog number
Voluette Ampule breaker 10 mL	each	2196800
Phosphate, Standard Solution, 10 mg/L	946 mL	1420416
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate; Standard Solution, 100 mg/L	100 mL	1436832
Phosphate; Standard Solution, 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate; Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01118

USEPA[1] PhosVer® 3 Method

Method 8048

(0.06 to 5.00 mg/L PO_4^{3-} or 0.02 to 1.60 mg/L P)

Test 'N Tube™ Vials

Scope and Application: For water, wastewater and seawater;

[1] USEPA accepted for reporting wastewater analysis. Procedure is equivalent to USEPA and Standard Method 4500-P E for wastewater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 314 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

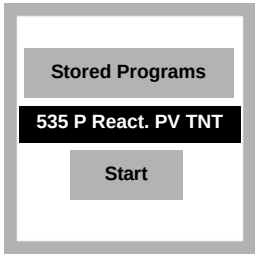
DR 3900, DR 3800, DR 2800 and DR 2700 only: Install the light shield in Cell Compartment #2 before performing this test.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

Description	Quantity
PhosVer® 3 Reagent Powder Pillow	1
Reactive Phosphorus Test 'N Tube Vial	1
Light Shield	1
Micro funnel	1
Pipet, TenSette®, 1–10 mL	1
Pipet tIps for TenSette Pipet	1
Test Tube Rack	varies

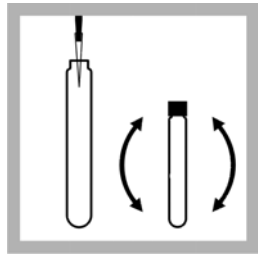
See *Consumables and replacement items* for reorder information.

PhosVer 3 Method, TNT



1. Select the test.

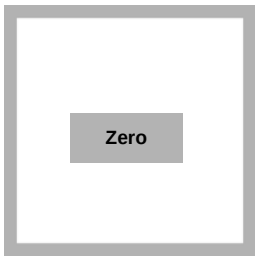
Insert an adapter if required (see *Instrument-specific information*).



2. Use a TenSette® Pipet to add 5.0 mL of sample to a Reactive Phosphorus Test 'N Tube Dilution Vial. Cap and mix.

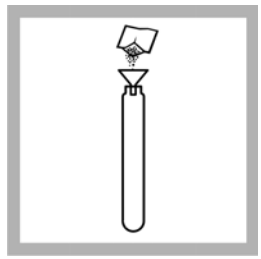
3. Wipe the outside of the vial with a damp towel, followed by a dry one, to remove fingerprints or other marks.

4. Insert the vial into the 16-mm round cell holder.

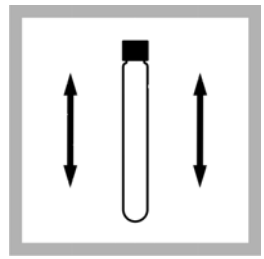


5. **ZERO** the instrument.

The display will show: 0.00 mg/L PO_4^{3-}



6. Using a funnel, add the contents of one PhosVer 3 Phosphate Powder Pillow to the vial.



7. Immediately cap the vial tightly and shake for at least 20 seconds. The powder will not dissolve completely.

8. Start the instrument timer.

A two-minute reaction period will begin. Read samples between two and eight minutes after adding the PhosVer 3 reagent.

9. Wipe the outside of the vial with a damp towel, followed by a dry one, to remove fingerprints or other marks.

10. When the timer expires, insert the vial into the 16 mm round cell.

READ the

Interferences

Table 315 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	All levels
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Sulfide	Greater than 6 mg/L. Remove sulfide interference as follows: **1.** Measure 25 mL of sample into a 50-mL beaker. **2.** Swirling constantly, add Bromine Water drop-wise until a permanent yellow color appears. **3.** Swirling constantly, add Phenol Solution drop-wise just until the yellow color disappears. Proceed with step 1 of the *PhosVer 3 Method, TNT* test.
Turbidity	Large amounts may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in this test.
- Analyze samples immediately after collection for best results.
- If prompt analysis is impossible, preserve samples up to 48 hours by filtering immediately and storing at 4 °C.
- Warm stored samples to room temperature before analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 2-mL Ampule Standard, 50-mg/L as PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **Options>More>Standard Additions**.

* See *Optional reagents and apparatus*.

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
6. Follow the *PhosVer 3 Method, TNT* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 3.0-mg/L phosphate standard solution

1. Use the 3.0-mg/L phosphate standard solution in place of the sample. Follow the *PhosVer 3 Method, TNT* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **Options>More>Standard Adjust**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
535	DR 5000	3.00 mg/L PO_4^{3-}	2.94–3.06 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}

Summary of method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Reactive Phosphorus Test 'N Tube™ Reagent Set (50 tests), includes:	—	—	2742545
PhosVer® 3 Phosphate Reagent Powder Pillows	1	50/pkg	2106046
Reactive Phosphorus Test 'N Tube Dilution Vials	1	50/pkg	NA[1]

[1] Not available separately

Required apparatus

Description	Quantity	Unit	Catalog number
Funnel, micro	1	each	2584335
Pipet, TenSette®, 1 to 10 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	1	50/pkg	2199796
Test Tube Rack	1–3	each	1864100

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, PouRite® Ampule, 50-mg/L as PO_4^{3-}, 2-mL	20/pkg	17120H
Phosphate Standard Solution, 50-mg/L	500 mL	17149
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL	256949
Phosphate Standard Solution, 3 mg/L as PO_4^{3-}	946 mL	2059716
Standard, Drinking Water, Mixed Parameter, Inorganic for F^-, NO_3, PO_4, SO_4	500 mL	2833049
Wastewater Effluent Standard, for mixed parameters: NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
PouRite Ampule breaker, 2 mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water 30 g/L	29 mL	221120
Hydrochloric Acid Solution, 6.0 N, 1:1	500 mL	88449
Phenol Solution 30 g/L	29 mL	211220
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Filter Paper, fold 12.5 cm	100/pkg	69257
Filter Funnel, Analytical, 65 mL	each	108367
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Beaker, 50-mL	each	50041H

[1] Other sizes are available

Optional standards

Description	Unit	Catalog number
Voluette Ampule breaker 10 mL	each	2196800
Phosphate, Standard Solution, 10 mg/L	946 mL	1420416
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate, Standard Solution, 50 mg/L, 10 mL Voluette Ampules	16/pkg	17110
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate, Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01116

Molybdovanadate Method[1] **Method 8114**

HR (1.0 to 100.0 mg/L PO_4^{3-}) **Test 'N Tube™ Vials**

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 316 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

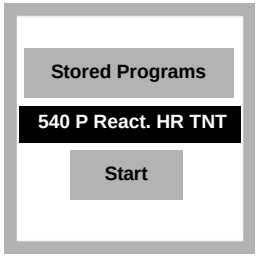
DR 3900, DR 3800, DR 2800 and DR 2700 only: Install the light shield in Cell Compartment #2 before performing this test.
Reagent blanks for each lot of reagents may be used more than once. At room temperature, the reagent blank is stable for up to three weeks.
The seven-minute reaction time in step 5 is for samples at 23 °C. For samples at 13 °C, wait 15 minutes. For samples at 33 °C, wait two minutes.
Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Refer to the current MSDS for safe handling and disposal information.

Collect the following items:

Description	Quantity
High Range Reactive Phosphorus Test 'N Tube Vials	1
Water, deionized	5 mL
Light Shield	1
Pipet, TenSette®, 1 to 10 mL	1
Pipet Tips, for TenSette Pipet	1
Test Tube Rack	1

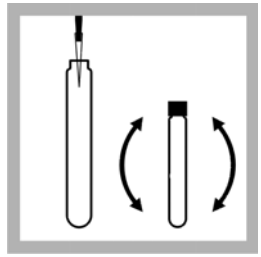
See *Consumables and replacement items* for reorder information.

Molybdovanadate method, TNT



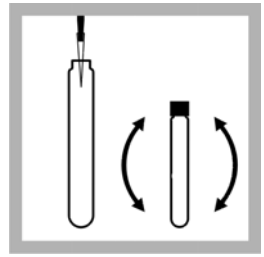
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Blank Preparation: Use a TenSette® Pipet to add 5.0 mL of deionized water to a Reactive High Range Phosphorus Test 'N Tube Vial.

Cap and invert to mix.



3. Prepared Sample: Use a TenSette Pipet to add 5.0 mL of sample to a Reactive High Range Phosphorus Test 'N Tube Vial.

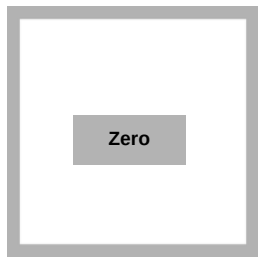
Cap and invert to mix.

4. Start the instrument timer.

A seven-minute reaction period will begin.

Read the sample within two minutes after the timer expires.

5. Wipe the reagent blank and insert it into the 16-mm round cell holder.

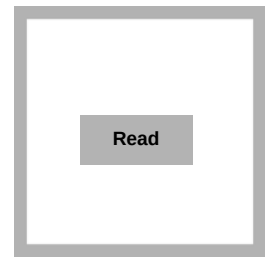


6. **ZERO** the instrument.

The display will show:

0.0 mg/L PO_4^{3-}

7. Wipe the prepared sample vial and insert it into the 16-mm round cell holder.



8. **READ** the results in mg/L PO_4^{3-}.

Interferences

The *Interfering substances* table lists interference types and levels. The *Noninterfering substances at low concentrations (less than 1000 mg/L)* table shows substances that do not interfere in concentrations less than 1000-mg/L.

Table 317 Interfering substances

Interfering substance	Interference level
Arsenate	Only interferes if the sample is heated.[1]
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Only interferes if the sample is heated.[1]
Sulfide	Causes a negative interference. Remove interference as follows: **1.** Measure 25 mL of sample into a 50-mL beaker. **2.** Add Bromine Water[2] drop-wise with constant swirling until a permanent yellow color develops. **3.** Add Phenol Solution[2] drop-wise until the yellow color just disappears. Proceed with step 3.
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause a negative interference.
Temperature, Cold (less than 20 °C)	Causes a negative interference.
Temperature, Hot (greater than 25 °C)	Causes a positive interference.

[1] Gentle warming of the sample to room temperature will not cause this substance to interfere.

[2] See *Optional reagents and apparatus*.

Table 318 Noninterfering substances at low concentrations (less than 1000 mg/L)

Pyrophosphate	Tetraborate	Benzoate
Citrate	Lactate	Formate
Oxalate	Tartrate	Salicylate
Al^{3+}	Selenate	Mg^{2+}
Ca^{2+}	Ba^{2+}	Sr^{2+}
Li^{+}	Na^{+}	K^{+}
NH^{4+}	Cd^{2+}	Mn^{2+}
NO_3^{-}	NO_2^{-}	SO_4^{2-}
SO_3^{2-}	Pb^{2+}	Hg^{+}
Hg^{2+}	Sn^{2+}	Cu^{2+}
Ni^{2+}	Ag^{+}	U
Zr^{4+}	AsO_3^{-}	Br^{-}
CO_3^{2-}	ClO_4^{-}	CN^{-}
IO_3^{-}	Fe^{3+}	SiO_4^{4-}

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in this test.
- For best results, analyze the samples immediately after collection.
- If prompt analysis is impossible, preserve the samples for up to 48 hours by filtering immediately and storing at 4 °C.
- Bring the stored sample to room temperature before analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- 10-mL Voluette® Ampule of Phosphate Standard Solution, 500-mg/L PO_4^{3-}
- 1:1 Hydrochloric acid solution
- Deionized water
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. Clean the glassware with 1:1 hydrochloric acid solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean the glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select standard additions from the instrument menu:**OPTIONS>MORE>STANDARD ADDITIONS**.
4. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
5. Open the standard solution ampule.
6. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
7. Follow the *Molybdovanadate method, TNT* test procedure using 5 mL of each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

* See *Optional reagents and apparatus*.

- 50-mg/L PO_4^{3-} standard

1. Use the 50-mg/L PO_4^{3-} standard solution in place of the sample. Follow the *Molybdovanadate method, TNT* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
540	50.0 mg/L PO_4^{3-}	49.1–50.9 mg/L PO_4^{3-}	0.7 mg/L PO_4^{3-}

Summary of method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, yellow molybdovanadophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 420 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
High Range Reactive Phosphorus Test 'N Tube™ Reagent Set, includes:	—	50 vials	2767345
(1) Reactive High Range Phosphorus Test 'N Tube Vials[1]	1	50/pkg	—
(2) Water, deionized	5 mL	100 mL	27242

[1] Not available separately.

Required apparatus

Description	Quantity	Unit	Catalog number
Pipet, TenSette®, 1 to 10 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	1	50/pkg	2199796
Test Tube Rack	1	each	1864100

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 50-mg/L, as PO_4^{3-}	500 mL	17149
Phosphate Standard Solution, Voluette® ampule, 500-mg/L as PO_4^{3-}, 10-mL	16/pkg	1424210
Wastewater Influent Standard for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide	500 mL	10649
Bromine Water, 30 g/L	29 mL	221120
Cylinder, graduated mixing, 25 mL	each	189640
Hydrochloric Acid Solution 6.0 N, 1:1	500 mL	88449
Phenol Solution, 30 g/L	29 mL	211220
Dropper, LDPE, 0.5–1.0 mL	20/pkg	2124720
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
pH Paper, 0 - 14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Filter Paper, fold 12.5 cm	100/pkg	69257
Filter Funnel, Analytical, 65 mL	each	108367
Beaker, 50-mL	each	50041H -
Finger cots	2/pkg	1464702

Optional standards

Description	Unit	Catalog number
Phosphate, Standard Solution, 3 mg/L	946 mL	2059716
Phosphate, Standard Solution, 10 mg/L	946 mL	1420416
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate, Standard Solution, 30 mg/L	946 mL	1436716
Phosphate, Standard Solution, 50 mg/L, 10 mL Voluette Ampules	16/pkg	17110
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 500 mg/L; 10 mL Voluette Ampules	16/pkg	1424210
Phosphate, Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Phosphorus, Total

DOC316.53.01121

USEPA[1]
PhosVer® 3 with Acid Persulfate Digestion Method

Method 8190

0.06 to 3.50 mg/L PO_4^{3-} or
0.02 to 1.10 mg/L P

Test 'N Tube™ Vials

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted for reporting wastewater analyses (Standard Methods 4500 P-E).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 319 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
The test range for total phosphate is limited to 0.06 to 3.5 mg/L PO_4^{3-}. Values greater than 3.5 mg/L may be used to estimate dilution ratios, but should NOT be used for reporting purposes. If the value is greater than 3.5 mg/L, dilute the sample and repeat the digestion and the colorimetric test.
Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Refer to the current MSDS for safe handling and disposal instructions.

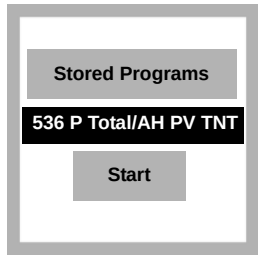
Collect the following items:

Description	Quantity
Total Phosphorus Test ‘N Tube™ Reagent Set	1
Deionized water	varies
DRB200 Reactor	1
Funnel, micro	1
Light Shield or Adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 1 to 10 mL, plus tips	1
Test Tube Rack	1

See *Consumables and replacement items* for reorder information.

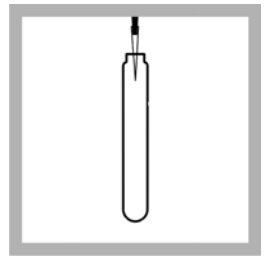
PhosVer 3, acid persulfate digestion

1. Turn on the DRB200 Reactor. Preheat to 150 °C.

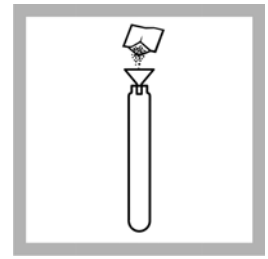


2. Select the test.

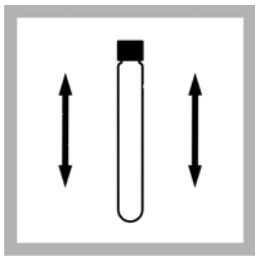
Insert an adapter if required (see *Instrument-specific information*).



3. Use a TenSette® Pipet to add 5.0 mL of sample to a Total Phosphorus Test Vial.



4. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.

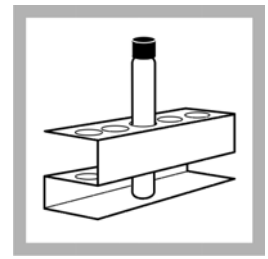


5. Cap tightly and shake to dissolve.

6. Insert the vial into the DRB200. Close the protective cover.

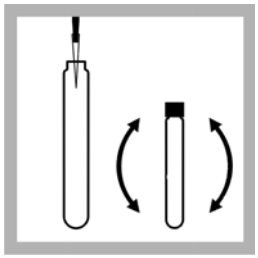
7. Set the instrument timer to 30 minutes and start.

A 30-minute heating period will begin.



8. When the timer expires, carefully remove the hot vial from the reactor. Insert it in a test tube rack and cool to room temperature.

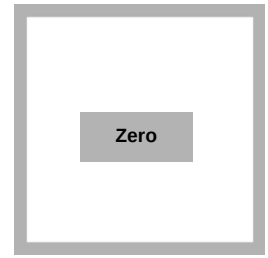
PhosVer 3, acid persulfate digestion (continued)



9. Use a TenSette Pipet to add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial. Cap and mix.

10. Wipe the outside of the vial with a damp cloth followed by a dry one.

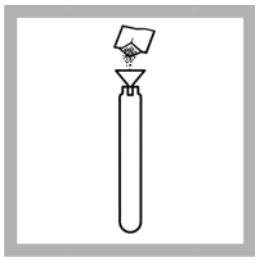
11. Insert the vial into the 16 mm cell holder.



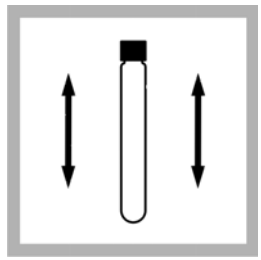
12. **ZERO** the instrument.

The display will show:

0.00 mg/L PO_4^{3-}



13. Use a funnel to add the contents of one PhosVer 3 Powder Pillow to the vial.



14. Immediately cap tightly and shake to mix for 20–30 seconds.

The powder will not dissolve completely.

15. Start the instrument timer.

A two-minute reaction period will begin.

Read the sample within 2–8 minutes after the timer expires.

16. After the timer expires, wipe the outside of the vial with a wet towel, then a dry one. Insert the prepared sample vial into the 16 mm cell.

READ the results in mg/L PO_4^{3-}.

Interferences

Table 320 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Silica	Greater than 50 mg/L

Table 320 Interfering substances (continued)

Interfering substance	Interference level
Silicate	Greater than 10 mg/L
Sulfide	Greater than 90 mg/L
Turbidity or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in this test.
- Analyze the samples immediately for the most reliable results.
- If prompt analysis is not possible, samples may be preserved up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric Acid* (about 2 mL per liter) and storing at 4 °C.
- Warm stored samples to room temperature and neutralize with 5.0 N Sodium Hydroxide* before analysis.
- Correct test results for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 10-mL Ampule Standard, 50-mg/L as PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders, (3)

1. Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select standard additions from the instrument menu **OPTIONS>MORE>STANDARD ADDITIONS**.
4. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
5. Open the standard solution ampule.
6. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
7. Use a 5-mL aliquot of the spiked sample in place of the sample. Follow the *PhosVer 3, acid persulfate digestion* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.

* See *Optional reagents and apparatus*.

8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate standard solution, 3.0-mg/L

1. Use the 3.0 mg/L phosphate standard solution in place of the sample. Follow the *PhosVer 3, acid persulfate digestion* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
536	3.00 mg/L PO_4^{3-}	2.93–3.07 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}

Summary of method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total Phosphorus Test 'N Tube™ Reagent Set, 50 tests, includes:	—	—	2742645
PhosVer® 3 Phosphate Reagent Powder Pillows	1	50/pkg	2106046
Potassium Persulfate Powder Pillows	1	50/pkg	2084766
Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	2743042
Total and Acid Hydrolyzable Test Vials[1]	1	50/pkg	—
Water, deionized	varies	100 mL	27242

[1] Not sold separately

Required apparatus

Description	Quantity	Unit	Catalog number
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001

Required apparatus

Description	Quantity	Unit	Catalog number
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Light shield, DR 3900	1	each	LZV849
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Pipet, TenSette®, 1.0 to 10 mL	1	each	1970010
Pipet Tips for TenSette Pipet 19700-10	1	250/pkg	2199725
Test Tube Rack	1	each	1864100

Recommended standards

Description	Unit	Catalog number
Drinking Water Standard, Mixed Parameter, Inorganic for F-, NO_3, PO_4, SO_4	500 mL	2833049
Phosphate Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as PO_4^{3-}	16/pkg	17110
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL	256949
Phosphate Standard Solution, 3 mg/L as PO_4^{3-}	946 mL	2059716
Wastewater Standard, Effluent Inorganics, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25 mL	each	189640
Pipet, volumetric, Class A, 2.00 mL	each	1451536
Hydrochloric Acid Solution, 6.0 N, 1:1	500 mL	88449
Sodium Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
Pipet, TenSette® Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
pH Paper, 0–14 pH range	100/pkg	2601300
Deionized Water	4 L	27256
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Finger cots	2/pkg	1464702

Optional standards

Description	Unit	Catalog number
Phosphate, Standard Solution, 10 mg/L	946 mL	1420416
Phosphate, Standard Solution, 15 mg/L	100 mL	1424342
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210

Optional standards

Description	Unit	Catalog number
Phosphate, Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Phosphorus, Total, Digestion

DOC316.53.01112

USEPA[1] Acid Persulfate Digestion Method[2]

Method 8190

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted for wastewater analyses when used with the ascorbic acid (PhosVer 3) method.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater* 4500-P B & E

Test preparation

Before starting the test:

Rinse all glassware with 1:1 hydrochloric acid. Rinse again with deionized water.

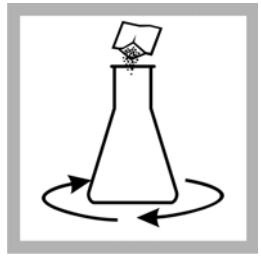
Collect the following items:

Description	Quantity
Potassium Persulfate Powder Pillows	1
Sodium Hydroxide Solution, 5.0 N	2 mL
Sulfuric Acid Solution, 5.25 N	2 mL
Water, deionized	varies
Cylinder, graduated, 25-mL	1
Flask, Erlenmeyer, 125-mL	1
Hot Plate	1

See *Consumables and replacement items* for reorder information.

Acid Persulfate Digestion

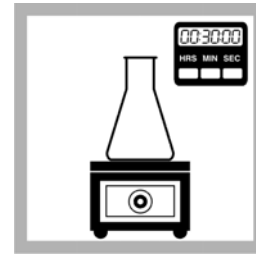
17. Use a graduated cylinder to measure 25 mL of sample. Pour the sample into a 125-mL Erlenmeyer flask.



18. Add the contents of one Potassium Persulfate Powder Pillow.

Swirl to mix.

19. Use a 1-mL calibrated dropper to add 2.0 mL of 5.25 N Sulfuric Acid Solution to the flask.

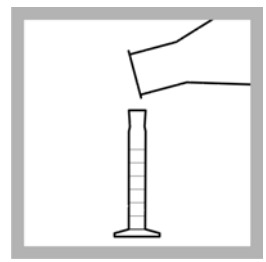


20. Place the flask on a hot plate. Boil gently for 30 minutes. Do not boil dry.

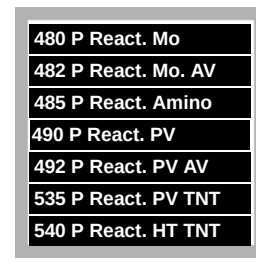
Concentrate the sample to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.

21. Cool the sample to room temperature.

22. Use a 1-mL calibrated dropper to add 2.0 mL of 5.0 N Sodium Hydroxide Solution to the flask. Swirl to mix.



23. Pour the sample into a 25-mL graduated cylinder. Adjust the volume to 25 mL with deionized water rinsings from the flask.



24. Proceed with a reactive phosphorus test of the expected total phosphorus concentration range.

Extend the color development time to 10 minutes for the PhosVer 3 (ascorbic acid) method.

Interferences

Table 321 Interfering substances

Interfering substance	Interference level
Alkaline or highly buffered samples	It may be necessary to add additional acid in step 19 to drop the pH of the solution below 1.
Turbidity	Use 50 mL of sample and double the reagent quantities. Use a portion of the reacted sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure.

Sample collection, preservation and storage

- Analyze the samples immediately for the most reliable results.
- If prompt analysis is not possible, samples may be preserved up to 28 days.
- To preserve samples. adjust the pH to 2 or less with Concentrated Sulfuric Acid* (about 2 mL per liter). Store at 4 °C.
- Warm the sample to room temperature and neutralize with 5.0 N Sodium Hydroxide before analysis.
- Correct test results for volume additions.

Summary of method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphate by heating with acid and persulfate. Organically bound phosphates are thus determined indirectly by subtracting the result of an acid hydrolyzable phosphorus test from the total phosphorus result.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determining the phosphorus content of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is USEPA accepted for NPDES reporting.

The following reagents and apparatus are required in addition to those required for the active phosphorus test.

* See *Optional reagents*.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Potassium Persulfate Powder Pillows	1	100/pkg	245199
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL MDB	245032
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL MDB	244932
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 25-mL	1	each	50840
Flask, Erlenmeyer, 125-mL	1	each	50543
Hot Plate, 7” x 7” digital, 115 VAC	1	each	2881500
Hot Plate, Stirrer, 7” x 7” digital, 230 VAC	1	each	2881602

Optional reagents

Description	Unit	Catalog number
Sodium, Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
pH paper, 0–14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, –10 to 225°C	each	2635700
Sampling bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Hydrochloric Acid, 6.0 N	500 mL	88449

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Edition 7

Phosphorus, Total

DOC316.53.01123

Molybdovanadate Method with Acid Persulfate Digestion[1]

Method 10127

HR (1.0 to 100.0 mg/L PO_4^{3-})

Test 'N Tube™ Vials

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*, (4500 B-C)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 322 Instrument-specific information

Instrument	Light shield
DR 6000	—
DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
Reagent blanks can be used more than once, but should not be used more than one day.
The final samples will contain molybdenum. In addition, the final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Refer to the current MSDS for safe handling and disposal instructions.

Collect the following items:

Description	Quantity
Total High Range Phosphorus Test 'N Tube™ Reagent Set	1
DRB200 Reactor, 15 x 16 mm	1
Light Shield or Adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 1 to 10 mL, plus tips	1
Test Tube Rack	1
Funnel, Micro	1

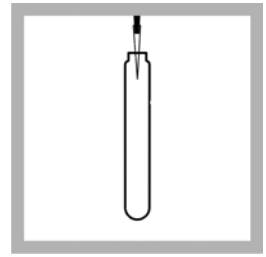
See *Consumables and replacement items* for reorder information.

Molybdovanadate method, acid persulfate digestion

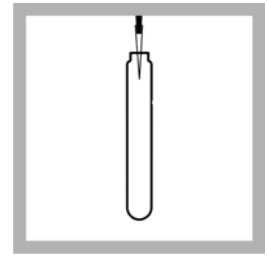
1. Turn on the DRB200 Reactor. Heat to 150 °C.

2. Select the test.

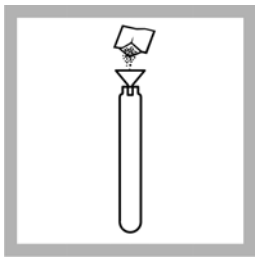
Insert an adapter if required (see *Instrument-specific information*).



3. Blank Preparation: Use a TenSette® Pipet to add 5.0 mL of deionized water to a Total Phosphorus Test 'N Tube Vial.



4. Prepared Sample: Use a TenSette Pipet to add 5.0 mL of sample to a Total Phosphorus Test 'N Tube Vial.



5. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow to each vial.

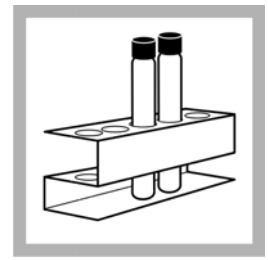
Cap tightly and shake to dissolve.

6. Insert the vials in the DRB 200 Reactor.

Close the protective cover.

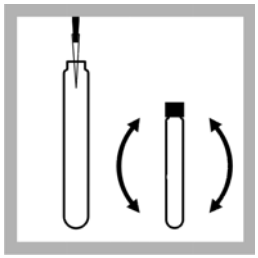
7. Set the instrument timer to 30 minutes and start the timer.

A 30-minute heating period will begin.



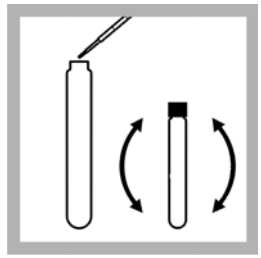
8. After the timer expires, carefully remove the hot vials from the reactor. Insert them in a test tube rack and allow to cool to room temperature (18–25 °C).

Molybdovanadate method, acid persulfate digestion (continued)



9. Use a TenSette Pipet to add 2.0 mL of 1.54 N sodium hydroxide to each vial.

Cap and invert to mix.



10. Use a polyethylene dropper to add 0.5 mL of Molybdovanadate Reagent to each vial.

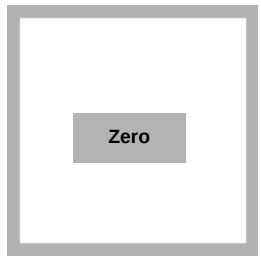
Cap and invert to mix.

11. Start the instrument timer.

A 7-minute reaction period will begin. Read the sample between seven and nine minutes after adding the Molybdovanadate Reagent.

12. Wipe the vials with a damp towel, followed by a dry one, to remove fingerprints or other marks.

13. When the timer expires, insert the blank into the 16-mm cell holder.

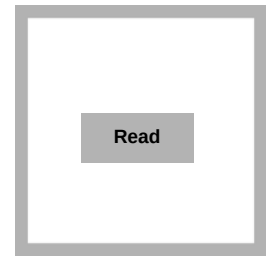


14. **ZERO** the instrument.

The display will show:

0.0 mg/L PO_4^{3-}

15. Insert the prepared sample into the 16-mm cell holder.



16. **READ** the results in mg/L PO_4^{3-}.

Interferences

Sample turbidity may cause inconsistent results in the test because the acid present in the reagents may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.

The *Interfering substances* table shows interference levels and types. The *Noninterfering substances, (at concentrations less than 1000 mg/L)* table shows substances that do not interfere in concentrations less than 1000 mg/L.

Table 323 Interfering substances

Interfering substance	Interference level
Arsenate	Causes positive interference if the sample is warm when the molybdovanadate reagent is added (after the digestion).[1] Cool the sample after digestion before adding reagent.
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.

Table 323 Interfering substances (continued)

Interfering substance	Interference level
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Causes positive interference if the sample is warm when the molybdovanadate reagent is added (after the digestion). Cool the sample after digestion before adding reagent.
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause a negative interference.
Temperature, Cold (less than 18 °C)	Cause a negative interference.
Temperature, Hot (greater than 25 °C)	Causes a positive interference. Post-digestion samples should be brought to room temperature (18–25 °C) before the addition of the Molybdovanadate Reagent or sodium hydroxide.

[1] Gentle warming of the sample to reach room temperature will not cause this substance to interfere.

Table 324 Noninterfering substances, (at concentrations less than 1000 mg/L)

Pyrophosphate	Tetraborate	Selenate	Benzoate
Citrate	Oxalate	Lactate	Tartrate
Formate	Salicylate	Al^{3+}	Fe^{3+}
Mg^{2+}	Ca^{2+}	Ba^{2+}	Sr^{2+}
Li^{+}	Na^{+}	K^{+}	NH_4^{+}
Cd^{2+}	Mn^{2+}	NO_3^{-}	NO_2^{-}
SO_4^{2-}	SO_3^{2-}	Pb^{2+}	Hg^{+}
Hg^{2+}	Sn^{2+}	Cu^{2+}	Ni^{2+}
Ag^{+}	U^{4+}	Zr^{4+}	AsO_3^{-}
Br^{-}	CO_3^{2-}	ClO_4^{-}	CN^{-}
IO_3^{-}	SiO_4^{4-}	—	

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning the glassware used in this test.
- Analyze samples immediately after collection for best results.
- If prompt analysis is impossible, preserve samples up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric Acid* (about 2 mL per liter) and storing at 4 °C.
- Warm stored samples to room temperature and neutralize with 5.0 N Sodium Hydroxide* before analysis.
- Correct the test results for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- 10-mL Voluette® Ampule of Phosphate Standard Solution, 500 mg/L as PO_4^{3-}
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select standard additions from the instrument menu **OPTIONS>MORE>STANDARD ADDITIONS**
4. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
5. Open the standard solution ampule.
6. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample in 25 mL mixing cylinders.
7. Follow the *Molybdovanadate method, acid persulfate digestion* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate standard solution, 50-mg/L

1. Use the 50-mg/L phosphate standard solution in place of the sample. Follow the *Molybdovanadate method, acid persulfate digestion* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
542	50 mg/L PO_4^{3-}	49.4–50.6 mg/L PO_4^{3-}	0.7 mg/L PO_4^{3-}

Summary of method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, yellow molybdovanadophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 420 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total High Range Phosphorus Test 'N Tube™ Reagent Set, includes:	—	50 vials	2767245
(1) Molybdovanadate Reagent[1]	0.5 mL	25 mL	—
(1) Potassium Persulfate powder Pillows	1	50/pkg	2084766
(1) Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	2743042
(1) Total Phosphorus Test Vials[1]	1	50/pkg	—
(2) Water, deionized	5 mL	100 mL	27242

[1] Not available separately.

Required apparatus

Description	Quantity	Unit	Catalog number
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Dropper, 0.5 and 1.0 mL marks, plastic	1	20/pkg	2124720
Light shield, DR 3900	1	each	LZV849
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Pipet, TenSette®, 1 to 10 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	1	250/pkg	2199725
Test Tube Rack	1	each	1864100
Funnel, micro	1	each	2584335

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, Voluette® ampule, 500-mg/L as PO_4^{3-}, 10-mL	16/pkg	1424210
Phosphate Standard Solution, 50-mg/L as PO_4^{3-}	500 mL	17149
Wastewater Standard, Influent Inorganics for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833149
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Hydrochloric Acid Solution, 6.0 N, 1:1	500 mL	88449
Sodium Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
Molybdovanadate Reagent	100 mL	2076032
Pipet Tips, for TenSette Pipet 1970010[1]	50/pkg	2199796
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
pH Paper, 0–14 pH range	100/pkg	2601300
Deionized Water	4 L	27256
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Finger cots	2/pkg	1464702
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Cylinder, mixing, 25 mL	each	189640
Funnel, micro	each	2584335

[1] Other sizes are available

Optional standards

Description	Unit	Catalog number
Phosphate, Standard Solution, 30 mg/L	946 mL	1436716
Phosphate, Standard Solution, 100 mg/L	100 mL	1436832
Phosphate, Standard Solution, 50 mg/L, 10 mL Voluette Ampules	16/pkg	17110
Phosphate, Standard Solution, 500 mg/L	100 mL	1424232

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Edition 7

Potassium

DOC316.53.01127

Tetraphenylborate Method **Method 8049**

0.1 to 7.0 mg/L **Powder Pillows**

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 325 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

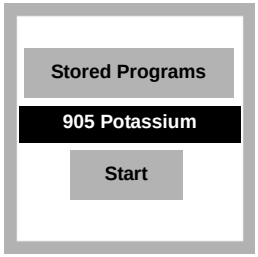
Program # 905 has a calibration curve for potassium; however, due to potential variation between lots of Potassium 3 Reagent, it is recommended that a new calibration for each lot of reagent is performed to obtain best accuracy. Prepare and store the calibration as directed under *Calibration*.
Filter highly colored or turbid samples before analysis.
Refer to a current MSDS for pollution prevention and waste management information.
After the test, clean the cells with soap and a brush.

Collect the following items:

Description	Quantity
Potassium Reagent 1 Powder Pillow	1
Potassium Reagent 2 Powder Pillow	1
Potassium Reagent 3 Powder Pillow	1
Clippers	1
Cylinder, mixing, 25-mL	1
Flask, volumetric, 100-mL Class A	8
Pipet, TenSette®, 1–10 mL, plus tips	varies
Sample Cells (see *Instrument-specific information*)	2
Water, deionized	varies

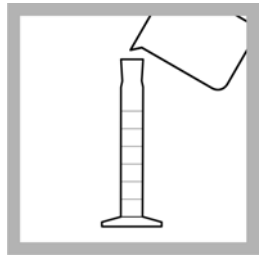
See *Consumables and replacement items* for reorder information.

Tetraphenylborate method for powder pillows

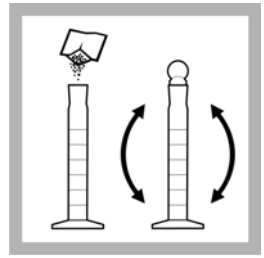


1. Select the test.

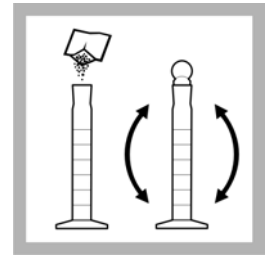
Insert an adapter if required (see *Instrument-specific information*).



2. Fill a graduated mixing cylinder with 25 mL of sample.



3. Add the contents of one Potassium 1 Reagent Pillow. Add the contents of one Potassium 2 Reagent Pillow. Stopper and invert several times to mix.

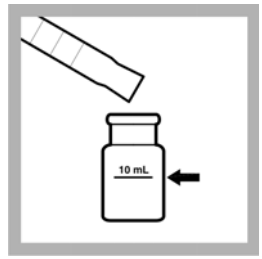


4. Add the contents of one Potassium 3 Reagent Pillow after the solution clears. Stopper and shake the solution for 30 seconds.

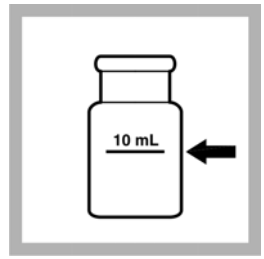
A white turbidity will form if potassium is present.

5. Start the instrument timer.

A three-minute reaction period will begin.

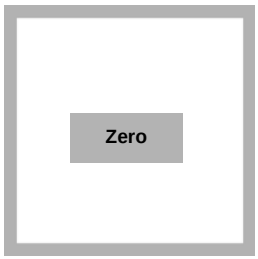


6. Prepared Sample: Pour at least 10-mL of the solution from the cylinder into a sample cell.



7. Blank Preparation: When the timer expires, fill the second sample cell with 10 mL of unreacted sample.

8. Wipe the blank and insert it into the cell holder.

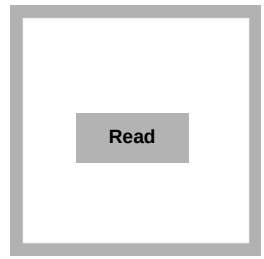


9. **ZERO** the instrument.

The display will show:

0.0 mg/L K

10. Within seven minutes after the timer expires, wipe the prepared sample and insert it into the cell holder.



11. **READ** the results in mg/L K.

Interferences

The substances in the *Interfering substances* table have been tested and will not interfere at or below the levels stated. If these substances are present at higher levels, conduct interference studies at the higher levels to determine if the substance interferes.

Table 326 Interfering substances

Interfering substance	Interference level
Ammonium Nitrogen	15 mg/L as N
Calcium	7000 mg/L as $CaCO_3$
Chloride	15,000 mg/L
Magnesium	6000 mg/L as $CaCO_3$

Sample collection, preservation and storage

- Collect samples in acid-washed plastic bottles.
- Adjust the pH to 2 or less with Nitric Acid (about 2 mL per liter)*.
- Preserved samples may be stored at least six months at room temperature.
- Before analysis, adjust the pH to 4–5 with 5.0 N Sodium Hydroxide*.
- Do not measure pH in the sample container with a pH electrode, as this will introduce potassium from the filling solution. Use pH Paper* or pour off sample and test pH in a separate beaker.
- Correct the test result for volume additions.

Accuracy check

Important Note: *This procedure is applicable only to Stored Program 905 and not to User Programs.*

Standard additions method (sample spike)
Required for accuracy check:

- Potassium Voluette® Ampule Standard, 250-mg/L K
- Ampule breaker
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.

* See *Optional reagents and apparatus*.

6. Follow the *Tetraphenylborate method for powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.

7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Calibration

Standard preparation

An approximate calibration curve is preprogrammed within Program 905. For improved accuracy, a new calibration should be performed with each new lot of reagents. Prepare calibration standards containing 1, 2, 3, 4, 5, 6, 7 and 8 mg/L potassium as follows:

1. Into eight different 100-mL Class A volumetric flasks, pipet 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mL of the 100-mg/L Potassium Standard Solution using class A glassware or TenSette Pipet.

2. Dilute to the mark with deionized water. Mix thoroughly.

3. Use deionized water for the 0-mg/L potassium standard.

User-entered Program

Refer to the user manual for instrument-specific information on user-entered programs.

1. Access the User Program feature.

2. For the initial potassium calibration, assign a new program number.

3. Enter a name for the potassium test.

4. Set up the following parameters

   - Program Type: Single wavelength
   - Units: mg/L
   - Wavelength l (nm): 650
   - Concentration Resolution: 0.1
   - Chemical Form: K
   - Calibration: Read Standards

5. Set up the following parameters:

   - Upper Limit: On, 8.0
   - Lower Limit: On, -0.2
   - Timer 1: Timer 3:00
   - Press Calibration: C=a + bA >Edit>OK

6. Enter the concentrations for the calibration, starting with 0.0, in the left column.

7. When all standard concentrations have been entered, navigate to the 0.0 line.

8. Insert the cell containing the blank (deionized water) and zero the instrument.

9. Perform the potassium test on each standard and insert the first prepared standard into the cell holder. Navigate to the line corresponding to this standard concentration. Read the result. Repeat for each standard concentration.

10. If the graph result is acceptable, exit the program. It may be possible to obtain a better fit to the data by reading another curve. The curve which results in the highest r2 value is generally the best fit. After selection of the best curve, exit the program.

11. Save the calibration.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
905	5.0 mg/L K	4.7–5.3 mg/L K	0.1 mg/L K

Summary of method

Potassium in the sample reacts with sodium tetraphenylborate to form potassium tetraphenylborate, an insoluble white solid. The amount of turbidity produced is proportional to the potassium concentration. Test results are measured at 650.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Potassium Reagent Set:	—	—	2459100
Potassium Reagent 1 Powder Pillow	1	25/pkg	1432198
Potassium Reagent 2 Powder Pillow	1	25/pkg	1432298
Potassium Reagent 3 Powder Pillow	1	100/pkg	1432399
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers	1	each	96800
Cylinder, mixing, 25-mL	1	each	189640
Flask, volumetric, 100-mL Class A	8	each	1457442
Pipet, TenSette®, 1–10 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	varies	50/pkg	2199796
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Potassium Standard Solution, 10-mL Voluette® Ampule, 250 mg/L	16/pkg	1479010
Potassium Standard Solution, 100-mg/L	500 mL	2351749
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Nitric Acid, 1:1	500 mL	254049
Sodium Hydroxide, 5.0 N	50 mL	245026
pH Paper, 0–14 pH range	100/pkg	2601300

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet Tips, for TenSette® Pipet 1970010[1]	250/pkg	2199725
Pipet, TenSette, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Brush, test tube	each	69000
Liqui-Nox detergent	946 mL	2088153

[1] Other sizes are available

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Edition 7

Quaternary Ammonium Compounds

DOC316.53.01128

Direct Binary Complex Method **Method 8337**

0.2 to 5.0 mg/L as CTAB **Powder Pillows**

Scope and Application: For cooling tower water and pool/spa water

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 327 Instrument-specific information

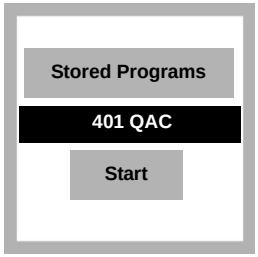
Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Collect the following items:

Description	Quantity
QAC Reagent 1 Powder Pillows	2 pillows
QAC Reagent 2 Powder Pillows	2 pillows
Bottle, square, with 25 mL mark	2
Clippers, for opening powder pillows	1
Sample Cells, 1-inch, 10 mL	2

See *Consumables and replacement items* for reorder information.

Direct Binary Complex Method



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. Blank Preparation: Fill one 25-mL mixing bottle with 25 mL of deionized water.

3. Prepared Sample: Fill another mixing bottle with 25 mL of sample.

4. Add the contents of one QAC Reagent 1 Powder Pillow to each bottle.

5. Swirl the bottles to dissolve the reagent.

Do not shake! Shaking creates air bubbles that interfere with test results.

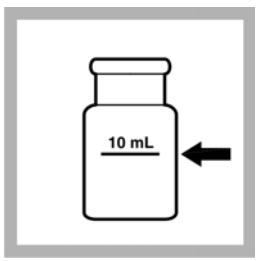
6. Add the contents of one QAC Reagent 2 Powder Pillow to each bottle.

7. Swirl the bottles to dissolve the reagent. **Do not shake.**

A purple color will form if a quaternary ammonium compound is present

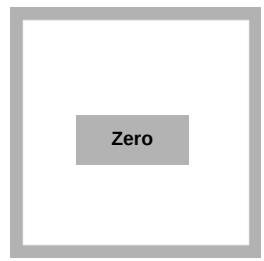
8. Start the instrument timer.

A two-minute reaction period will begin.



9. Pour at least 10 mL of the solutions from the bottles into the sample cells.

10. When the timer expires, insert the blank into the cell holder.



11. ZERO the instrument.

The display will show:

0.0 mg/L CTAB

12. Insert the prepared sample into the cell holder.

READ the results in mg/L CTAB (cetyl-trimethylammonium bromide).

Interferences

Interference studies were conducted by preparing a CTAB standard solution of approximately 3 mg/L as well as a solution of the potential interference. The constituent was said to interfere when the resulting concentration changed by 10%. The *Interfering substances* table shows interfering substances and levels. The *Noninterfering substances* table shows substance that do not interfere up to the tested concentrations.

After several samples have been analyzed, the sample cells may exhibit a build-up of a pink or purple color. A rinse with 1.0 N Sodium Hydroxide Solution followed by an Alconox™ detergent wash and deionized water rinse will eliminate the build-up when it occurs.

Table 328 Interfering substances

Interfering substance	Interference level
Calcium (as $CaCO_3$)	Positive interference above 1350 mg/L
Chlorine, HOCl and OCl^-	Positive interference above 7 mg/L
Cyanuric acid	Negative interference above 70 mg/L
Igepal™ nonionic surfactant	Positive interference above 3 mg/L
Iodine, I_3^-	Positive interference above 3 mg/L
Iron, Fe^{3+}	Positive interference above 80 mg/L
Liquimine™ 14–P, filming amine	Positive interference above 1825 mg/L
Magnesium, Mg^{2+}	Positive interference above 1350 mg/L
Niaproof™ anionic surfactant	Negative interference above 11 mg/L
Polyacrylic acid	Negative interference above 16 mg/L
Sodium lauryl sulfate	Negative interference above 8 mg/L
Sodium polyphosphate	Positive interference above 1325 mg/L
Tribenzylamine	Positive interference above 7 mg/L
Triton X-100™ nonionic surfactant	Positive interference above 4 mg/L
Urea	Positive interference above 8 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment. Adjust the sample pH between 3 and 5 by using a pH meter or pH paper and adding dropwise an appropriate amount of acid or base such as 1.0 N Sulfuric Acid Standard Solution or 1.0 N Sodium Hydroxide Standard Solution. If significant volumes of acid or base are used, a volume correction should be made.

Table 329 Noninterfering substances

Non-interfering Substance	Highest Concentration Tested (mg/L)
Silica, SiO_2	400
Potassium alum, $AlKS_2O_8$	500
Sodium thiosulfate, $Na_2S_2O_3$	30

Sample collection, preservation and storage

- Collect samples in glass bottles that have been rinsed several times with sample before final sample filling.
- Do not use plastic containers; plastic adsorbs QACs.
- Acidify the sample to a pH of less than 2.
- Store at 4 ± 2 °C.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- QAC Standard Solution, 100-mg/L CTAB
- TenSette Pipet and tips
- Mixing bottles (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
6. Follow the *Direct Binary Complex Method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- QAC Standard, 100-mg/L as CTAB
- Deionized water
- 100-mL Class A volumetric flask
- Class A 5 mL volumetric pipet and pipet bulb

1. Prepare a 5.0 mg/L CTAB standard solution as follows:
   a. Pipet 5.0 mL of QAC Standard, 100-mg/L as CTAB, into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use this solution in place of the sample. Follow the *Direct Binary Complex Method* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
401	DR 5000	3.0 mg/L CTAB	2.7–3.3 mg/L CTAB	0.04 mg/L CTAB

Summary of method

The test method makes use of a colorimetric chemistry in which a quaternary ammonium compound reacts with an indicator to produce a color change from pale pink to vivid purple. The test is conducted in a stabilized, acid-buffered solution containing a masking agent to eliminate potential interferences. This test is applicable to the monitoring of QACs in swimming pools and cooling towers. Test results are measured at 575 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Quaternary Ammonium Compounds Reagent Set (100 tests), includes:	—	—	2459200
(4) QAC Reagent 1 Powder Pillows	2 pillows	50/pkg	2401066
(8) QAC Reagent 2 Powder Pillows	2 pillows	25/pkg	2401268

Required apparatus (powder pillows)

Description	Quantity	Unit	Catalog number
Bottle, square, with 25 mL mark	2	each	1704200
Clippers, for opening powder pillows	1	each	96800
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
QAC Standard Solution, 100-mg/L as CTAB	100 mL	2415342
Water, deionized	4 liters	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Alconox™ detergent	1.8 kg	2088000
Sodium Hydroxide Standard Solution, 1.0 N	100 mL	104532
Sodium Hydroxide Solution, 1.0 N	1000 mL	104553
Sulfuric Acid Standard Solution, 1.0 N	100 mL	127032
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Flask, Class A volumetric, 100 mL	each	1457442
Pipet, Class A volumetric, 5 mL	each	1451537

[1] Other sizes are available

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Edition 7

Salinity

DOC316.53.001180

Mercuric Nitrate Method

Method 10073

0–100 ppt[1] Salinity

Digital Titrator

Scope and Application: For seawater and brackish water

[1] parts per thousand (ppt)

Test preparation

Before starting the test:

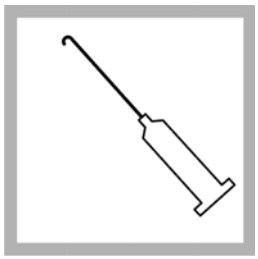
ppt salinity x 569 = mg/L chloride (Cl^-).
ppt salinity x 940 = mg/L sodium chloride (NaCl)
Use the TitraStir apparatus for best results
Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. Refer to the MSDS for more information on proper disposal of these materials.

Collect the following items:

Description	Quantity
Diphenylcarbazone Reagent Powder Pillows	1
Deionized Water	varies
Digital Titrator	1
Mercuric Nitrate Titration Cartridge, 2.570 N	varies
Syringe, 3-cc, Luer lock tip	1
Vial with 2-, 5-, 10-, 15-, 20- and 25-mL marks	1

See *Consumables and replacement items* for reorder information.

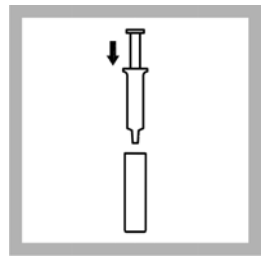
Iodate-Iodide Method



1. Insert a clean delivery tube into the Mercuric Nitrate Titration Cartridge.

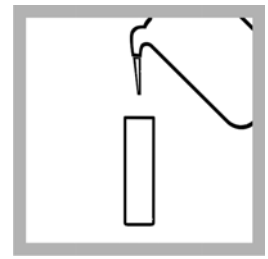
Attach the cartridge to the titrator body.

2. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

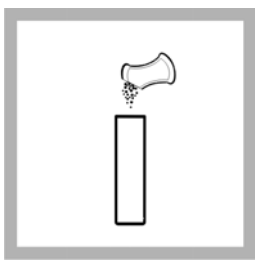


3. Use the 3-mL syringe to collect a 2.0-mL water sample. Add to the vial, provided.

Note: *2 mL = 2 cc*

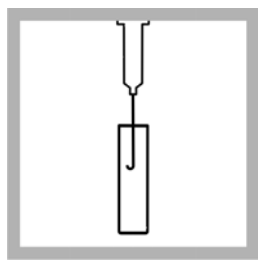


4. Fill the vial to the 10-mL mark with deionized water.



5. Add the contents of one Diphenylcarbazone Reagent Powder Pillow to the vial and mix.

A small amount of undissolved reagent will not affect results.



6. Titrate the sample with mercuric nitrate until the color changes from yellow to light pink.

Record the number of digits.

7. Calculate sample salinity in parts per thousand:

Digits Required x 0.1 = ppt Salinity

Summary of method

The mercuric nitrate method of chloride analysis has become popular due to the sharp yellow to pinkish-purple end point of diphenylcarbazone. A single, stable powder has been developed, combining the color indicator with an appropriate buffer to establish the correct pH.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Diphenylcarbazone Reagent Powder Pillows	100/pkg	96799
Mercuric Nitrate Titration Cartridge, 2.570 N	each	2393701
Water, Deionized	4 L	27256
Chloride Standard, 12,500 mg/L as Cl-, (22 ppt Salinity) 10 mL AMP	16/pkg	1425010
Sodium Chloride standard, 10,246 mg/L as NaCl, 100 mL AMP	10.9 ppt salinity	2307442

Required apparatus

Description	Unit	Catalog number
Delivery tubes w/ 180 ° hook	each	1720500
Delivery tubes w/ 90° hook	each	4157800
Digital Titrator2	each	1690001
Syringe, 3-cc, Luer lock tip	each	4321300
Vial with 2-, 5-, 10-, 15-, 20- and 25-mL marks	each	219300
Voluette breaker	each	2196800

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Edition 7

Selenium

DOC316.53.01129

Diaminobenzidine Method[1]

Method 8194

0.01 to 1.00 mg/L

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 330 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2612602	Fill line faces right
DR 5000	2612602	Fill line faces user
DR 3900	2612602	Fill line faces user
DR 3800, DR 2800, DR 2700	2612602	Fill line faces right

Before starting the test:

Distillation is required for determining total selenium. See *Distillation* at the end of the procedure. Use the distillate as the sample in step 2.
Acetone[1] is a suitable solvent for removing toluene from glassware after results are measured.
Toluene (F005) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Water saturated with toluene, toluene solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent wastes. Refer to the current MSDS for safe disposal and handling information.
If there are visible water bubbles on the bottom of the cell, decant the top portion into a clean, dry cell prior to reading the sample.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

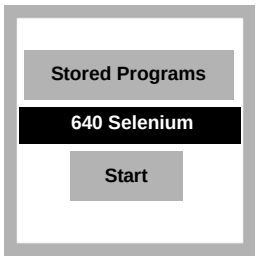
[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
Buffer Solution, sulfate type, pH 2.0	10 mL
Cotton Ball	1
Cylinder, Graduated: 50- and 100-mL	1 of each
Diaminobenzidine, tetrahydrochloride	0.1 g
Distillation reagents and apparatus	—
Dropper, 0.5 and 1.0 mL marks, one glass and one plastic	1 of each
Flask, Erlenmeyer, 500-mL	2
Funnel, separatory, 250 mL	2
Hot Plate, 7-inch	1
Pipet, volumetric, 5-mL, plus safety bulb filler	1
Potassium Hydroxide Standard Solution, 12 N	4 mL
Ring support (3-inch) and stand	2
Sample Cells (see *Instrument-specific information*)	2
Spoons, measuring, 0.2 and 0.05 g	1 of each
TitraVer® Hardness Reagent	0.4 g
Toluene, ACS	60 mL
Water, Deionized	100 mL

See *Consumables and replacement items* for reorder information.

Diaminobenzidine method

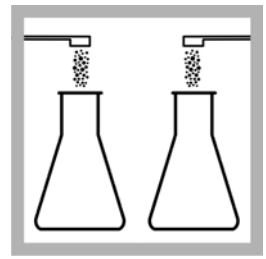


1. Select the test.

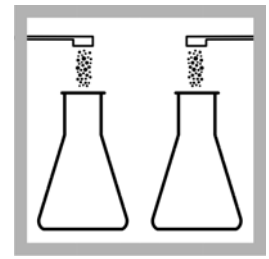
Insert an adapter if required (see *Instrument-specific information*).

2. Measure 100 mL of deionized water into a 500-mL Erlenmeyer flask. Label the flask “blank”.

Measure 100 mL of sample into a 500-mL Erlenmeyer flask. Label the flask “sample”.



3. Add a 0.2-g spoonful of TitraVer® Hardness Reagent to each flask. Swirl to mix.



4. Add a 0.05-g spoonful of diaminobenzidene tetrahydrochloride to each flask. Swirl to mix.

Diaminobenzidine method (continued)

5. If you have not distilled the sample, add 5.0 mL of Buffer Solution, sulfate type, pH 2.0 to each flask. Swirl to mix.

If the sample has been distilled, adjust the pH of the distillate to pH 2.7 ± 0.2 using 5 N Sodium Hydroxide Standard Solution. Adjust the blank to pH 2.7 ± 0.2 using 5.25 N Sulfuric Acid Standard Solution.

6. Heat each flask on a hot plate. Bring the contents to a gentle boil.

7. Start the instrument timer.

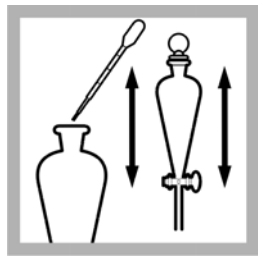
A five-minute reaction period will begin. Continue to boil the contents gently during this time period.

A yellow color will develop if selenium is present.

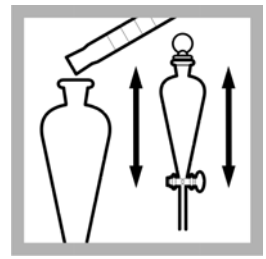
8. When the timer expires, remove both flasks. Cool to room temperature using a water bath.

Do not boil more than one minute after the timer expires.

9. Transfer the contents of each flask to separate 250-mL separatory funnels. Label the funnels “blank” and “sample”. Place each flask in a support ring on a stand.



10. Add 2.0 mL of 12 N Potassium Hydroxide Standard Solution to each funnel using a calibrated 1.0-mL plastic dropper. Stopper. Shake each funnel to mix.



11. Add 30-mL of toluene to each funnel. Stopper. Swirl and invert each funnel, then open the stopcock to vent the funnel. Close the stopcock. Repeat twice with each funnel.

Use toluene only with adequate ventilation.

12. Start the instrument timer.

A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the blank.

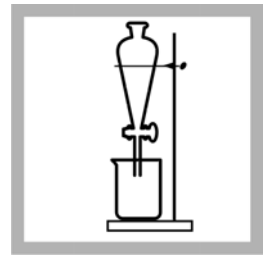
Diaminobenzidine method (continued)

13. Start the instrument timer.

A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the sample.

14. Start the instrument timer.

A four-minute reaction period will begin.



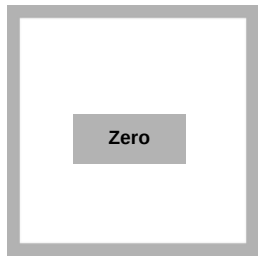
15. When the timer expires, remove the stopper and drain the lower water layer from each funnel and discard.

Complete steps 16–19 within five minutes after the timer expires. The developed color is stable, but should be measured as soon as possible.

16. Insert a cotton plug into the delivery tube of each separatory funnel. Slowly drain the toluene into respective sample cells labeled “blank” and “sample”. Stopper the sample cells.

Filtering the toluene through dry, absorbent cotton will remove water or suspended particles.

17. Wipe the blank and insert it into the cell holder.

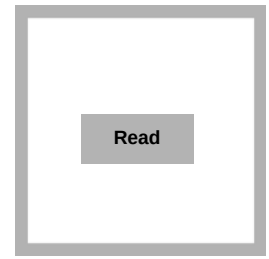


18. **ZERO** the instrument.

The display will show:

0.00 mg/L Se

19. Wipe the prepared sample and insert it into the cell holder.



20. **READ** the results in mg/L Se.

Interferences

There are no positive inorganic interferences with this method. Any other interferences can be removed by distilling the sample.

Table 331 Interfering substances

Interfering substance	Interference level
Ferric iron	Up to 2.5 mg/L. Distill sample to eliminate interference.
Manganese	Will not interfere.
Strong oxidizing agents (i.e., iodine, bromine or chlorine)	Can react with the indicator to give low results. Distill sample to eliminate interference.

Sample collection, preservation and storage

- Collect samples in clean glass or plastic containers.
- Adjust the pH to 2 or less with Nitric Acid* (about 1.5 mL per liter).
- Preserved samples can be stored for up to six months at room temperature.
- Correct the test result for volume additions.

Distillation

CAUTION
Always perform this procedure under a fume hood.

This distillation involves the use of a strong acid and oxidizer at high temperatures. To avoid personal injury, observe all laboratory safety precautions when operating the distilling apparatus.

1. Measure 500 mL of sample into a 1000-mL beaker.
2. Add 1 mL of Methyl Orange Indicator Solution. Stir with a glass rod.
3. Use a dropper to add 0.1 N Hydrochloric Acid Standard Solution dropwise until the solution becomes pink. Then add an additional 2 mL.
4. Use a pipet to add 5.0 mL Calcium Chloride Solution. Mix well.
5. Use a dropper to add 1-g/L Potassium Permanganate Standard Solution drop-wise until the solution is purple.
6. Place the beaker on a hot plate. Evaporate the solution to approximately 250 mL. Periodically add 1-g/L Potassium Permanganate Solution to keep the solution purple.
7. Any precipitate formed at this step is manganese dioxide and may be ignored.
8. Cool the solution. While cooling, set up the distillation apparatus for the general purpose distillation as shown in the distillation manual.
9. Pour the treated sample solution into the distillation flask. Add a stirring bar to the flask.
10. Pipet 5.0 mL of 0.1 N Sodium Hydroxide Standard Solution into the flask. Turn the stirrer power switch to ON. Set the stir control to 5.
11. Turn on the water and adjust so a constant flow is maintained through the condenser. Set the heat control to 10.
12. When only a few milliliters are left in the distillation flask, turn the power switch off. The distillate in the Erlenmeyer flask may be discarded.

CAUTION
Perform step 13 under a fume hood.

13. When the flask has cooled, add 50 mL of 19.2 N Sulfuric Acid Standard Solution to the flask. Add 10 grams of Potassium Bromide† to the flask.
14. Fill a 250-mL beaker to the 75-mL mark with deionized water. Place it under the drip tube. Elevate the beaker with a laboratory jack so the tube extends below the level of the water.

* See *Optional reagents and apparatus*.

† Purchase Potassium Bromide from a local chemical supplier.

15. Add 1.0 mL of 30% hydrogen peroxide solution to the flask. Turn the stir control to 5 and the heat control to 10. Cap the distillation flask.
16. Heat the distillation flask until the yellow color is gone from the complete distillation apparatus, including the J-tube and condenser. Remove the beaker from under the drip tube.
17. Turn off the heater switch. When the J-tube and condenser have cooled, rinse them with deionized water. Add the washings to the 250-mL beaker. Total volume in the beaker should be approximately 100 mL.
18. Add the Phenol Solution drop-wise to the distilled sample to discharge the bromine color (a white precipitate of tribromophenol will form).
19. Allow the precipitate to settle. Using a dropper, collect about 5 mL of the clear, colorless distillate and transfer to a test tube.
20. Test the solution for completeness of precipitation by adding 2 drops of Phenol Solution. If the solution becomes cloudy or white precipitate forms, residual bromine is still present (proceed to next step). If no cloudiness occurs, the sample is ready for analysis.
21. Transfer the 5-mL aliquot back to the beaker and continue to add Phenol Solution until no turbidity is formed in subsequent 5-mL aliquots.
22. Transfer the entire sample into a 500-mL volumetric flask. Rinse the beaker with deionized water and add to the flask.
23. Dilute to volume with deionized water, stopper and mix well. The distillate is now ready for analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Selenium Standard Solution, 1000 mg/L
- 100-mL volumetric flask
- 10 mL volumetric pipet and pipet bulb
- Deionized water
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare a 100-mg/L selenium standard solution:
   a. Pipet 10 mL of 1000-mg/L selenium standard solution into a 100-mL volumetric flask.
   b. Dilute to volume with demineralized water.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of the prepared standard to three 100-mL portions of fresh sample.

6. Follow the *Diaminobenzidine method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.

7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 1000 mg/L Selenium Standard Solution
- Deionized water
- 100-mL volumetric flask
- 200-mL volumetric flask
- 500-mL Erlenmeyer flask
- Volumetric pipet
- TenSette Pipet

1. Prepare a 0.5 mg/L Selenium standard solution:

   a. Prepare a 100-mg/L selenium standard solution: Pipet 10 mL of 1000-mg/L selenium standard solution into a 100-mL volumetric flask. Dilute to volume with demineralized water.

   b. Pipet 1.00 mL of the 100-mg/L solution into a 200-mL volumetric flask. Dilute to volume with deionized water.

   c. Transfer 100 mL of the standard into a 500-mL Erlenmeyer flask

2. Use this solution in place of the sample. Follow the *Diaminobenzidine method* test procedure.

3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
640	0.50 mg/L Se	0.47–0.53 mg/L Se	0.01 mg/L Se

Summary of method

An EDTA masking agent is added to the sample to remove interferences such as iron prior to the test. The addition of a sulfate buffer adjusts the sample to the optimum pH of 1 to 2. Under these conditions, diaminobenzidine reacts with all selenium present as selenite (Se^{4+}) to give a yellow-colored piazselenol complex which is extracted and the color intensity measured colorimetrically. Selenium present as Se^{2+} and Se^{6+} is not detected unless the sample is distilled. Test results are measured at 420 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Selenium Reagent Set (100 tests[1]), includes:			2244200
(1) Buffer Solution, sulfate type, pH 2.0	10 mL	500 mL	45249
(1) Diaminobenzidine, tetrahydrochloride	0.1 g	5 g	706222
(2) Potassium Hydroxide Standard Solution, 12 N	4 mL	100 mL	23032
(1) TitraVer® Hardness Reagent, ACS	0.4 g	100 g	20426
(1) Toluene, ACS	60 mL	4 L	1447017
Water, deionized	100 mL	4 L	27256

[1] This test requires a reagent blank. The number of tests shown refers to any combination of samples and blanks.

Required apparatus

Description	Quantity	Unit	Catalog number
Cotton Balls, absorbent	1	100/pkg	257201
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Dropper, 0.5 & 1.0 mL marks, glass	1	5/pkg	1419705
Dropper, 0.5 & 1.0 mL marks, plastic	1	20/pkg	2124720
Flask, Erlenmeyer, 500-mL	2	each	50549
Funnel, separatory, 250-mL	2	each	52046
Hot Plate, 7x7-inch, 120 VAC	1	each	2881500
Hot plate stirrer 7x7-inch, 220-240 VAC	1	each	2881602
Pipet, volumetric, 5-mL	1	each	1451537
Pipet filler, safety bulb	1	each	1465100
Ring, support, (3-inch) 83-mm	2	each	58000
Sample Cells, 1-inch square, 25 mL with stopper, matched pair	2	2/pkg	2612602
Spoon, measuring, 0.05-g	1	each	49200
Spoon, measuring, 0.2-g	1	each	63800
Support, ring stand, (5 x 8 inch) 127 x 203 mm	2	each	56300

Distillation reagents and apparatus

Description	Unit	Catalog number
Calcium Chloride Solution	1000 mL	42853
Hydrochloric Acid Standard Solution, 0.1 N	1000 mL	1481253
Hydrogen Peroxide, 30%, ACS	473 mL	14411
Methyl Orange Indicator Solution, (0.50-g/L)	500 mL	14849
Phenol Solution, 30-g/L	29 mL	211220
Potassium Permanganate Solution, 1-g/L	100 mL	1416442
Potassium Bromide, ACS grade[1]	—	—
Sodium Hydroxide Standard Solution, 0.100 N	1000 mL	19153

Distillation reagents and apparatus

Description	Unit	Catalog number
Sulfuric Acid Standard Solution, 19.2 N	500 mL	203849
Distillation Apparatus Set, general purpose	each	2265300
Distillation Apparatus Heater, 115 VAC	each	2274400
Distillation Apparatus Heater, 230 VAC	each	2274402
Beaker, 1000 mL	each	50053
Glass stirring rod	3/pkg	177001
Stir bar, magnetic	each	2095351
Beaker, 250 mL	each	50046H
Test tubes, 24 mL, 16 mm diameter	10/pkg	56524

[1] Purchase Potassium Bromide from a local chemical supplier

Recommended standards

Description	Unit	Catalog number
Selenium Standard Solution, 1000-mg/L	100 mL	2240742

Optional reagents and apparatus

Description	Unit	Catalog number
Acetone, ACS	500 mL	1442949
Nitric Acid, ACS	500 mL	15249
Sodium Hydroxide, 5.0 N	100 mL	245032
Sulfuric Acid, 5.25 N	100 mL	244932
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
pH Paper, 0–14 pH range	100/pkg	2601300
Flask, Class A volumetric, 100 mL	each	1457442
Flask, Class A volumetric, 200 mL	each	1457445
Pipet, Class A volumetric, 10 mL	each	1451538
Pipet, Class A volumetric, 1 mL	each	1451535

[1] Other sizes are available

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Edition 7

Silicomolybdate Method **Method 8185**

HR (1 to 100 mg/L) **Powder Pillows**

Scope and Application: For water and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 332 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Sample temperature should be 15–25 °C (59–77 °F)
This method is very sensitive to small differences in the measurement wavelength. For best results, use the Standard Adjust feature (as described in *Accuracy check*, *Standard solution method*) to optimize results on each instrument.

Collect the following items:

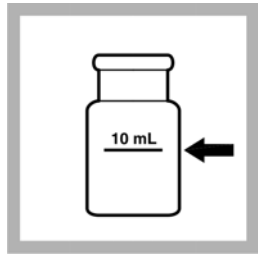
Description	Quantity
High Range Silica Reagent Set	1
Sample Cell (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

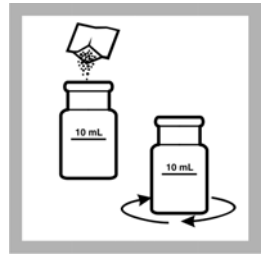
Silicomolybdate method for powder pillows

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Fill a sample cell with 10-mL of sample.



3. Prepared Sample: Add the contents of one Molybdate Reagent Powder Pillow for High Range Silica to the sample cell. Swirl until completely dissolved.

4. Add the contents of one Acid Reagent Powder Pillow for High Range Silica. Swirl to mix.

A yellow color will develop if silica or phosphorus is present.

5. Start the instrument timer.

A ten-minute reaction period will begin.

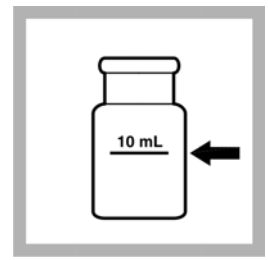
6. When the timer expires, add the contents of one Citric Acid Powder Pillow to the sample cell. Swirl to mix.

Any yellow color due to phosphorus is removed in this step.

7. Start the instrument timer.

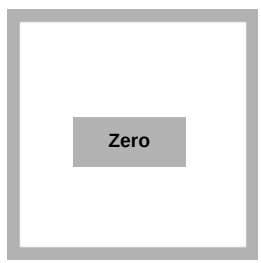
A two-minute reaction period will begin.

Perform steps 3–11 within three minutes after the timer expires.



8. Blank Preparation: Fill a second sample cell with 10 mL of the original sample.

9. Wipe the blank and insert the blank into the cell holder.

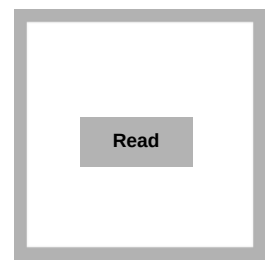


10. ZERO the instrument.

The display will show:

0 mg/L SiO_2

11. Wipe the prepared sample and insert the prepared sample into the cell holder.



12. READ the results in mg/L SiO_2.

Interferences

Occasionally a sample contains silica which reacts very slowly with molybdate. The nature of these “molybdate-unreactive” forms is not known. A pretreatment with Sodium Bicarbonate*, then Sulfuric Acid* will make these forms reactive to molybdate. The pretreatment is given in *Standard Methods for the Examination of Water and Wastewater* under Silica-Digestion with Sodium Bicarbonate. A longer reaction time with the sample and the molybdate and acid reagents (before adding citric acid) may help instead of the bicarbonate treatment.

Table 333 Interfering substances

Interfering substance	Interference level
Color	Eliminated by zeroing the instrument with the original sample.
Iron	High levels of Fe^{2+} and Fe^{3+} interfere.
Phosphate	Does not interfere below 50 mg/L PO_4^{3-}. At 60 mg/L PO_4^{3-}, a negative 2% interference occurs. At 75 mg/L PO_4^{3-}, the interference is negative 11%.
Sulfides (S^{2-})	All levels interfere.
Turbidity	Eliminated by zeroing the instrument with the original sample.

Sample collection, preservation and storage

- Collect samples in clean plastic bottles.
- Analyze samples as soon as possible after collection.
- If prompt analysis is not possible, store samples at 4 °C (39 °F) for up to 28 days.
- Warm stored samples to room temperature before analyzing.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Silica Standard Solution, 1000 mg/L
- TenSette Pipet and Pipet Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
6. Follow the *Silicomolybdate method for powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.

* See *Optional reagents and apparatus*.

7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Silica Standard Solution, 50-mg/L

1. Use the Silica Standard Solution, 50-mg/L in place of the sample. Use deionized water as the blank. Follow the *Silicomolybdate method for powder pillows* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
656	50 mg/L SiO_2	48–52 mg/L SiO_2	1.0 mg/L SiO_2

Summary of method

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Silica is then determined by measuring the remaining yellow color. Test results are measured at 452 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
High Range Silica Reagent Set for 10-mL samples (100 tests), includes:			2429600
Acid Reagent Powder Pillows for High Range Silica	1	100/pkg	2107469
Citric Acid Powder Pillows	1	100/pkg	2106269
Molybdate Reagent Powder Pillows for High Range Silica	1	100/pkg	2107369
Water, deionized	10 mL	4 L	27256

Recommended standards

Description	Unit	Catalog number
Silica Standard Solution, 50-mg/L	200 mL	111729
Silica Standard Solution, 1000-mg/L	500 mL	19449

Optional reagents and apparatus

Description	Unit	Catalog number
Sodium Bicarbonate	454 grams	77601
Sulfuric Acid 1.00 N	100 mL	127032
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700

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Edition 7

Heteropoly Blue Method[1] **Method 8186**

LR (0.010 to 1.600 mg/L as SiO_2) **Powder Pillows**

Scope and Application: For boiler and ultrapure water

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 334 Instrument-specific information

Instrument	Powder pillows	
	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

The four-minute reaction time in step 4 is for samples at 20 °C; for samples at 10 °C, wait eight minutes; for samples at 30 °C, wait two minutes.
The one-minute reaction time in step 6 is for samples at 20 °C; for samples at 10 °C, wait two minutes; for samples at 30 °C, wait 30 seconds.
If testing for very low levels of silica, use Method 8282.
For best results, use matched cells.

Collect the following items:

Description	Quantity
Amino Acid F Reagent Powder Pillows (for 10-mL sample)	1 pillow
Citric Acid Powder Pillows	2 pillows
Molybdate 3 Reagent Solution	1 mL
Sample Cells see *Instrument-specific information*	2

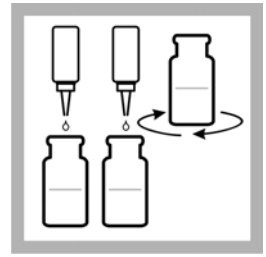
See *Consumables and replacement items* for reorder information.

Heteropoly Blue

1. Select the test.

2. Insert an adapter if required (*Instrument-specific information*).

Fill two sample cells (*Instrument-specific information*) with 10 mL of sample.



3. Add 14 drops of Molybdate 3 Reagent to each sample cell. Swirl to mix.

4. Start the timer. A four-minute reaction period will begin.

5. When the timer expires, add the contents of one Citric Acid Reagent Powder Pillow to each sample cell. Swirl to mix.

6. Start the timer. A one-minute reaction period will begin.

The destruction of possible phosphate interference occurs during this period.

7. Prepared Sample: When the timer expires, add the contents of one Amino Acid F Reagent Powder Pillow to one of the sample cells. Swirl to mix.

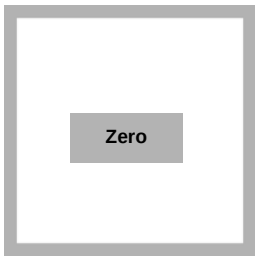
Blank Preparation: The sample without the Amino Acid F Reagent is the blank.

8. Start the timer.

A two-minute reaction period will begin.

A blue color will develop if silica is present.

Heteropoly Blue (continued)

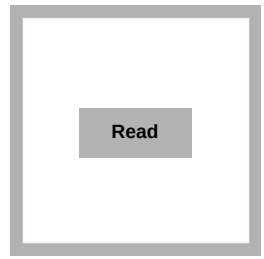


9. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show 0.000 mg/L SiO_2.

10. Wipe the prepared sample and insert it into the cell holder with the fill line facing the user.



11. **READ** the results in mg/L (SiO_2)

Interferences

Table 335 Interfering substances and levels

Interfering substance	Interference level
Color	Eliminated by zeroing the instrument with the original sample.
Iron	Large amounts interfere.
Phosphate	Does not interfere at levels less than 50 mg/L PO_4. At 60 mg/L PO_4, an interference of –2% occurs. At 75 mg/L PO_4 the interference is –11%.
Slow reacting forms of silica	Occasionally a sample contains silica which reacts very slowly with molybdate. The nature of these “molybdate-unreactive” forms is not known. A pretreatment with Sodium Bicarbonate, then Sulfuric Acid will make these forms reactive to molybdate. The pretreatment is given in *Standard Methods for the Examination of Water and Wastewater* under Silica-Digestion with Sodium Bicarbonate. A longer reaction time with the sample and the molybdate and acid reagents (before adding citric acid) may help instead of the bicarbonate pretreatment.
Sulfides	Interfere at all levels.
Turbidity	Eliminated by zeroing the instrument with the original sample.

Sample collection, preservation and storage

Collect samples in clean plastic bottles. Analyze samples as soon as possible after collection. If prompt analysis is not possible, store samples for up to 7 days by cooling to 4 °C (39 °F) or below. Warm samples to room temperature before analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Silica standard solution, 25 mg/L
- TenSette Pipet and Pipet tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu **OPTIONS>MORE>STANDARD ADDITIONS**.
3. A summary of the standard additions procedure will be displayed. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row.
4. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL, and 0.3 mL of standard to three 10-mL portions of fresh sample.
5. Follow the test procedure for each of the spiked samples starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
6. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Use a 1.00-mg/L SiO_2 Standard Solution in place of the sample. Perform the silica procedure.
2. To adjust the calibration curve using the reading obtained with the 1.00-mg/L Standard Solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
651	1.000 mg/L SiO_2	0.990–1.010 mg/L SiO_2	0.012 mg/L SiO_2

Summary of method

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. An Amino Acid is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration. Test results are measured at 815 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog Number
Low Range Silica Reagent Set (100 tests), includes:	—	—	2459300
Amino Acid F Reagent Powder Pillows (for 10-mL sample)	1	100/pkg	2254069
Citric Acid Powder Pillows	2	100/pkg	2106269
Molybdate 3 Reagent Solution	1 mL	50 mL	199526

Recommended Standard

Description	Unit	Catalog Number
Deionized Water	4 L	27256
Silica Standard Solution, 1-mg/L SiO_2	500 mL	110649
Silica Standard Solution, 25-mg/L as SiO_2	236 mL	2122531

Optional Reagents and Apparatus

Description	Unit	Catalog Number
Sodium Bicarbonate	454 g	77601
Sulfuric Acid, 1.00 N	1000 mL	127053
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips for TenSette Pipet 19700-01	50/pkg	2185696

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Edition 7

Heteropoly Blue Rapid Liquid Method[1] **Method 8282**

ULR(3 to 1000 µg/L as SiO_2) **Pour-Thru Cell**

Scope and Application: For testing trace levels of soluble silica in pure and ultrapure water

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 336 Instrument-specific information

Instrument	Pour-Thru Cell	Pour-Thru Cell Orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

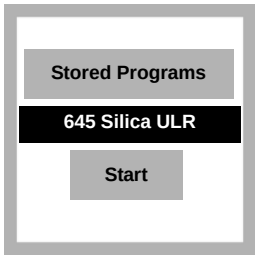
See the user manual for Pour-Thru Module installation instructions.
Clean the Pour-Thru cell and all labware as specified in *Labware*
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
See *Reagent preparation* for instructions on preparing the Amino Acid F Reagent.
The four-minute reaction time in step 10 is for samples at 20 °C; for samples at 10 °C, wait eight minutes; for samples at 30 °C, wait two minutes.
The one-minute reaction time in step 12 is for samples at 20 °C; for samples at 10 °C, wait two minutes; for samples at 30 °C, wait 30 seconds.
Make sure the Pour-Thru cell is completely seated in the sample cell compartment.

Collect the following items:

Description	Quantity
Amino Acid F Reagent Powder	varies
Amino Acid Reagent Dilution Solvent	varies
Citric Acid F Reagent	1 mL
Molybdate 3 Reagent	1 mL
Cylinder, graduated, 50-mL, poly	1
Dispenser, fixed volume, 1.0-mL, w/bottle	3
Flask, Erlenmeyer, 250-mL, PMP, w/cap	2
Pour-Thru Cell Module (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

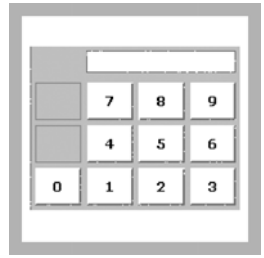
Heteropoly Blue Rapid Liquid method



1. Select the test.

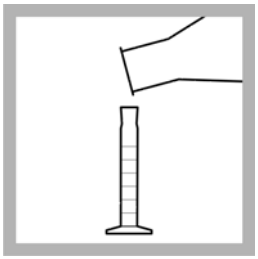
Insert an adapter if required (see *Instrument-specific information*).

2. Activate the reagent blank option to account for the Molybdate 3 reagent blank. The value is printed on the bottle label.

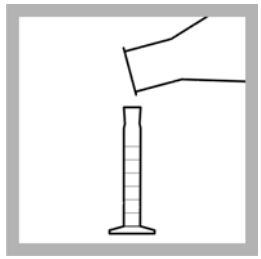


3. Use the numeric keypad on the instrument to manually adjust the reagent blank value.

4. Fill two clean 250-mL Erlenmeyer flasks to overflowing with sample.

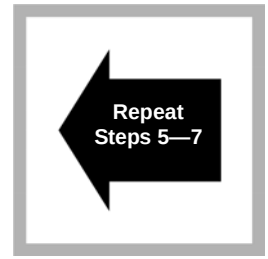


5. Fill a clean 50-mL plastic graduated cylinder with sample from one of the flasks; then discard the contents of the cylinder. Repeat three times.



6. Fill the rinsed cylinder to the 50-mL mark with sample from the same flask. Discard any remaining sample in the flask.

7. Pour the contents of the 50-mL cylinder back into the original flask.



8. Repeat steps 5 through 7 for the second flask containing sample.

Heteropoly Blue Rapid Liquid method (continued)

9. Add 1.0 mL of Molybdate 3 Reagent to each flask. Swirl to mix.

10. Start the instrument timer.

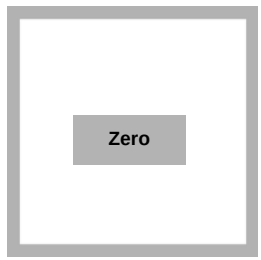
A four-minute reaction period will begin.

11. When the timer expires, add 1.0 mL of Citric Acid F Reagent to each flask. Swirl to mix.

12. Start the instrument timer.

A one-minute reaction period will begin. The destruction of possible phosphate interference occurs during this period.

13. When the timer expires, pour the contents of one flask into the Pour-Thru Cell.



14. After the flow stops, **ZERO** the instrument.

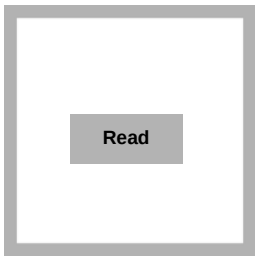
The display will show:

0 µg/L SiO_2

15. Add 1.0 mL of Amino Acid F Reagent to the remaining flask. Swirl to mix.

A faint blue color will develop if silica is present.

16. Wait at least 15 seconds, then pour the contents of the second flask into the Pour-Thru Cell.



17. **READ** the results in mg/L SiO_2.

18. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Table 337 Interfering substances

Interfering substance	Interference level
Color	Eliminated by zeroing the instrument with the blank (follow procedure).
Iron	Interferes at high levels.
pH (extreme)	Adjust pH to less than 7.
Phosphate (PO_4^{3-})	Interferes at levels greater than 50 mg/L PO_4^{3-}.
Sulfides	Interfere at all levels.
Turbidity	Eliminated by zeroing the instrument with the blank (follow procedure).

Sample collection, preservation and storage

- Use only plastic containers with tight-fitting closures. Do not use glass containers; they will contaminate the sample with silica.
- Soak sampling containers with a solution made of one part Molybdate 3 Reagent to 50 parts of high quality deionized water of low silica concentration. Fill the containers completely and let them stand for several hours. Rinse thoroughly with low-level silica water, drain and close. Repeat this cleaning periodically.
- Allow the sample stream to flow for 1–2 minutes before collection. Do not adjust the flow during the sampling period as this may introduce particulates.
- Rinse the container well with sample before collecting the portion for analysis.
- Analyze samples as soon as possible.

Reagent preparation

1. Dissolve the contents of one bottle of Amino Acid F Reagent Powder in one bottle of Amino Acid Reagent Dilution Solvent.
2. Install a bottle-top dispenser on this bottle, as well as on the Molybdate 3 Reagent and Citric Acid Reagent bottles.
3. Prepare smaller volumes of Amino Acid F Reagent by dissolving Amino Acid F Reagent Powder in Amino Acid F Reagent Solvent at a ratio of 11 grams per 100 mL of reagent solvent.

This prepared solution has limited stability; test routinely with the 1-mg/L (1000 µg/L) Silica Standard Solution to confirm performance.

Reduced sensitivity at high concentrations (1000 µg/L) indicates reagent instability. If the concentration is less than 950 µg/L, use fresh Amino Acid F Reagent Solution.

Labware

All containers used in this test must be cleansed thoroughly to remove any traces of silica. Use plastic containers for all analysis and storage because glass can contaminate the sample with silica. Small bottles or flasks with screw-type closures work well.

1. Clean containers by normal means (do not use phosphate detergents), then rinse with high quality deionized water of low-level silica concentration.
2. Soak for 10 minutes with a 1:50 dilution of Molybdate 3 Reagent in low-level silica water.
3. Rinse repeatedly with either low-level silica water or the sample before use. Keep containers tightly closed when not in use.

4. Fill the Pour-Thru Cell with this same mixture of Molybdate 3 and water and let stand for several minutes before use.
5. Rinse with low-level silica water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement.

1. Remove the color by rinsing with a 1:5 dilution of ammonium hydroxide and deionized water.
2. Rinse several times with deionized water.
3. Cover the Pour-Thru Cell when it is not in use.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- 1-mg/L (1000-µg/L) Silica standard
- TenSette Pipet and Pipet Tips.
- 250-mL plastic Erlenmeyer flasks (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS.**
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare three samples. Fill three plastic Erlenmeyer flasks with 50 mL of prepared sample.
5. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL and 0.6 mL of the 1-mg/L standard to each flask and mix thoroughly.
6. Follow the *Heteropoly Blue Rapid Liquid method* test procedure for each of the spiked samples, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 500-µg/L SiO_2 Standard Solution

1. Use a 500-µg/L SiO_2 Standard Solution in place of the sample. Follow the *Heteropoly Blue Rapid Liquid method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software:**OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
645	500 µg/L silica	496–504 µg/L silica	13 µg/L silica

Summary of method

A number of modifications are necessary to adapt the Low Range Silica method for analyzing trace levels in the Ultra Low Range method. It is absolutely necessary to use the one-inch Pour-Thru Cell and liquid reagents. The Pour-Thru Cell increases the reproducibility of the optics and reduces the instability of the readings that result from moveable sample cells. Liquid reagents produce more reproducible readings and lower blank values by eliminating slight turbidity that may remain when using powdered reagents. Use of liquid reagents in continuous monitors for silica provides a means of confirming the analyzer performance.

Silica and phosphate in the sample react with molybdate ions under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Amino Acid F Reagent is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration. Test results are measured at 815 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Rapid Liquid ULR Silica Reagent Set, includes:			2678500
Amino Acid F Reagent Powder	varies	55 g	2651155
Amino Acid Reagent Dilution Solvent	varies	475 mL	2353011
Citric Acid F Reagent	1 mL	500 mL	2254249
Molybdate 3 Reagent	1 mL	500 mL	199549

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 50-mL, poly	1	each	108141
Dispenser, fixed volume, 1.0-mL, w/bottle	3	each	2111302
Flask, Erlenmeyer, 250-mL, PMP, w/cap	2	each	2089846
Funnel, powder	1	each	2264467

Recommended standards

Description	Unit	Catalog number
Silica Standard Solution, 1-mg/L SiO_2	500 mL	110649
Silica Standard Solution, 500-µg/L SiO_2	3.78 L	2100817
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, 58%	500 mL	10649
Molybdate 3 Reagent	2.9 L	199503
Molybdate 3 Reagent	3.78 L	199517
Molybdate 3 Reagent	100 mL	199532
Molybdate 3 Reagent	1 L	199553
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet tips for TenSette Pipet 1970001	50/pkg	2185696
Sampling Bottle with cap, low density polyethylene, 500 mL	12/pkg	2087079
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700

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Edition 7

Heteropoly Blue Method[1] **Method 8282**

ULR(3 to 1000 µg/L as SiO_2) **Pour-Thru Cell**

Scope and Application: For testing trace levels of soluble silica in pure and ultrapure water

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 338 Instrument-specific information

Instrument	Pour-Thru Kit	Cell Orientation	Adapter
DR 6000	LQV175.99.20002	Arrow faces right	—
DR 5000	LZV479	—	—
DR 3900	LQV157.99.10002	Align cell flow arrows with arrows on cell compartment	—
DR 3800, DR 2800, DR 2700	5940400	1-inch (round) path aligned with arrow on the adapter	LZV585 (B)

Before starting the test:

See the user manual for Pour-Thru Module installation instructions.
Clean the Pour-Thru cell and all labware as specified in *Labware*.
Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
See *Reagent preparation* for instructions on preparing the Amino Acid F Reagent.
The four-minute reaction time in step 10 is for samples at 20 °C; for samples at 10 °C, wait eight minutes; for samples at 30 °C, wait two minutes.
The one-minute reaction time in step 12 is for samples at 20 °C; for samples at 10 °C, wait two minutes; for samples at 30 °C, wait 30 seconds.
Make sure the PourThru cell is completely seated in the sample cell compartment.

Collect the following items:

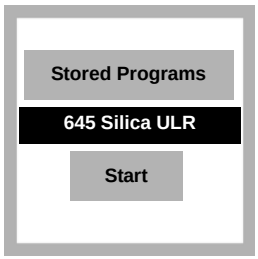
Description	Quantity
Amino Acid F Reagent Solution	1 mL
Citric Acid F Reagent	1 mL
Molybdate 3 Reagent	1 mL
Cylinder, graduated, 50-mL, poly	1
Flask, Erlenmeyer, 250-mL, PMP, with cap	2
Pipet, TenSette®, 0.1 to 1.0 mL with tips	1

Collect the following items:

Description	Quantity

See *Consumables and replacement items* for reorder information.

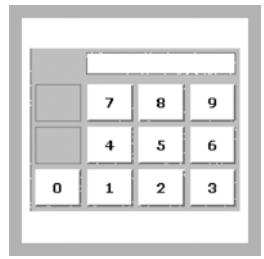
Heteropoly Blue method



1. Select the test.

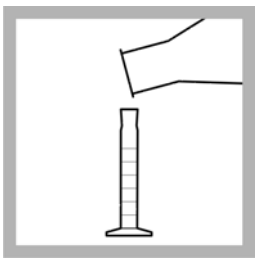
Insert an adapter if required (see *Instrument-specific information*).

2. Activate the reagent blank option to account for the Molybdate 3 reagent blank. The value is printed on the reagent blank.

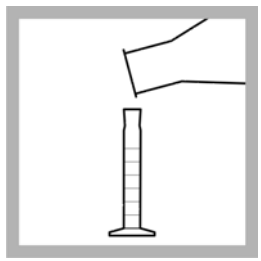


3. Use the numeric keypad on the instrument to manually adjust the reagent blank value.

4. Fill two clean 250-mL Erlenmeyer flasks to overflowing with sample.

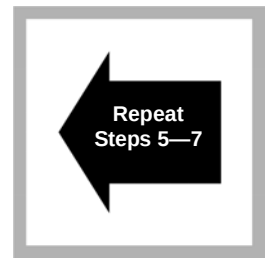


5. Fill a clean 50-mL plastic graduated cylinder with sample from one of the flasks; then discard the contents of the cylinder. Repeat three times.



6. Fill the rinsed cylinder to the 50-mL mark with sample from the same flask. Discard any remaining sample in the flask.

7. Pour the contents of the 50-mL cylinder back into the original flask.



8. Repeat steps 5 through 7 for the second flask containing sample.

Heteropoly Blue method (continued)

9. Use a TenSette® Pipet to add 1.0 mL of Molybdate 3 Reagent to each flask. Swirl to mix.

10. Start the instrument timer.

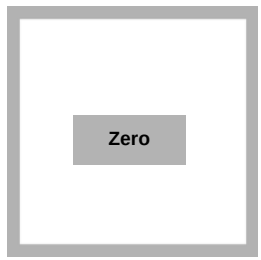
A four-minute reaction period will begin.

11. When the timer expires, add 1.0 mL of Citric Acid F Reagent to each flask. Swirl to mix.

12. Start the instrument timer.

A one-minute reaction period will begin. The destruction of possible phosphate interference occurs during this period.

13. When the timer expires, pour the contents of one flask into the Pour-Thru Cell.



14. After the flow stops, **ZERO** the instrument.

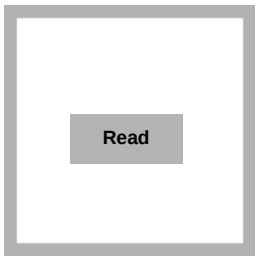
The display will show:

0 µg/L SiO_2

15. Add 1.0 mL of Amino Acid F Reagent to the remaining flask. Swirl to mix.

A faint blue color will develop if silica is present.

16. Wait at least 15 seconds, then pour the contents of the second flask into the Pour-Thru Cell.



17. **READ** the results in mg/L SiO_2.

18. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Table 339 Interfering substances

Interfering substance	Interference level
Color	Eliminated by zeroing the instrument with the blank (follow procedure).
Iron	Interferes at high levels.
pH (extreme)	Adjust pH to less than 7.
Phosphate (PO_4^{3-})	Interferes at levels greater than 50 mg/L PO_4^{3-}.
Sulfides	Interfere at all levels.
Turbidity	Eliminated by zeroing the instrument with the blank (follow procedure).

Sample collection, preservation and storage

- Use only plastic containers with tight-fitting closures. Do not use glass containers; they will contaminate the sample with silica.
- Soak sampling containers with a solution made of one part Molybdate 3 Reagent to 50 parts of high quality deionized water of low silica concentration. Fill the containers completely and let stand for several hours. Rinse thoroughly with low-level silica water, drain and close. Repeat this cleaning periodically.
- Allow the sample stream to flow for 1–2 minutes before collection. Do not adjust the flow during the sampling period as this may introduce particulates.
- Rinse the container well with sample before collecting the portion for analysis.
- Analyze samples as soon as possible.

Reagent preparation

Amino Acid F Reagent Solution is available in either 100-mL bottles or a package of 20 unit-dose ampules. The bottled reagent is stable for up to one year if the bottle is kept closed when not in use. The ampuled reagent is sealed under argon and is more stable with a shelf life greater than 1 year. Reduced sensitivity at high concentrations (1000 µg/L) indicates reagent instability. Check the bottled reagent on a routine basis by performing an analysis on a 1-mg/L (1000 µg/L) Silica Standard Solution. If the concentration is less than 950 µg/L, use a fresh bottle of Amino Acid F Reagent Solution.

Prepare larger or smaller volumes of Amino Acid F Reagent by dissolving Amino Acid F Reagent Powder in Amino Acid F Reagent Solvent at a ratio of 11 grams per 100 mL of reagent solvent. These reagents are available as the Amino Acid F Reagent Package. This prepared solution has limited stability; test routinely with the 1-mg/L Silica Standard Solution.

Labware

All containers used in this test must be cleansed thoroughly to remove any traces of silica. Use plastic containers for all analysis and storage because glass can contaminate the sample with silica. Small bottles or flasks with screw-type closures work well.

1. Clean containers by normal means (do not use phosphate detergents), then rinse with high quality deionized water of low-level silica concentration.
2. Soak for 10 minutes with a 1:50 dilution of Molybdate 3 Reagent in low-level silica water.
3. Rinse repeatedly with either low-level silica water or the sample before use. Keep containers tightly closed when not in use.
4. Fill the Pour-Thru Cell with this same mixture of Molybdate 3 and water and let stand for several minutes before use.
5. Rinse with low-level silica water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement.

1. Remove the color by rinsing with a 1:5 dilution of ammonium hydroxide and deionized water.
2. Rinse several times with deionized water.
3. Cover the Pour-Thru Cell when it is not in use.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- 1-mg/L (1000-µg/L) Silica standard
- TenSette Pipet and Pipet tips.
- 250-mL plastic Erlenmeyer flasks (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITION**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare three samples. Fill three plastic Erlenmeyer flasks with 50 mL of prepared sample.
5. Use the TenSette Pipet to prepare spiked samples: add 0.2 mL, 0.4 mL and 0.6 mL of the 1-mg/L standard to each flask and mix thoroughly.
6. Follow the *Heteropoly Blue method* test procedure for each of the spiked samples, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 500-µg/L SiO_2 Standard Solution

1. Use a 500-µg/L SiO_2 Standard Solution in place of the sample. Follow the *Heteropoly Blue method* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
645	DR 5000	500 µg/L silica	496–504 µg/L silica	13 µg/L silica

Summary of method

A number of modifications are necessary to adapt the Low Range Silica method for analyzing trace levels in the Ultra Low Range method. It is absolutely necessary to use the one-inch Pour-Thru Cell and liquid reagents. The Pour-Thru Cell increases the reproducibility of the optics and reduces the instability of the readings that result from moveable sample cells. Liquid reagents produce more reproducible readings and lower blank values by eliminating slight turbidity that may

remain when using powdered reagents. Use of liquid reagents in continuous monitors for silica provides a means of confirming the analyzer performance.

Silica and phosphate in the sample react with molybdate ions under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Amino Acid F Reagent is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration. Test results are measured at 815 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
ULR Silica Reagent Set (using Amino Acid F solution, 100 tests)	—	—	2553500
Includes: (2) 199532, (2) 2254232, (1) 2386442			
ULR Silica Reagent Set (using Amino Acid F ampules, 40 tests)	—	—	2581400
Includes: (1) 199532, (1) 2254232, (2) 2386420			
Amino Acid F Reagent Solution	1.0 mL	100 mL	2386442
OR			
Amino Acid F Reagent Solution, 1.2-mL Ampules	1	20/pkg	2386420
Citric Acid Reagent Solution	2 mL	500 mL	2254249
Molybdate 3 Reagent Solution	2.0 mL	500 mL	199549

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, 50-mL, poly	1	each	108141
Flask, Erlenmeyer, 250-mL, PMP, w/cap	2	each	2089846
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	1970001
Pipet Tips, for TenSette Pipet 19700-01	5	50/pkg	2185696

Recommended standards

Description	Unit	Catalog number
Silica Standard Solution, 1-mg/L SiO_2	500 mL	110649
Silica Standard Solution, 500-µg/L SiO_2	3.78 L	2100817
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, 58%	500 mL	10649
Molybdate 3 Reagent	2.9 L	199503
Molybdate 3 Reagent	3.78 L	199517
Molybdate 3 Reagent	100 mL	199532
Molybdate 3 Reagent	1 L	199553
PourRite® ampule breaker	each	2484600
Amino Acid F reagent package	each	2254117
Sampling Bottle with cap, low density polyethylene, 500 mL	12/pkg	2087079
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700

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Edition 7

Silver

DOC316.53.01134

Colorimetric Method **Method 8120**

(0.02 to 0.70 mg/L) **Powder Pillows**

Scope and Application: For water and wastewater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 340 Instrument-specific information

Instrumetn	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces user
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800 DR 2700	2495402	Fill line faces user

Before starting the test:

Digestion is required for samples with interferences. See *Digestion*.

For best results, measure a reagent blank value for each new lot of reagent (follow the procedure using deionized water in place of the sample). Subtract the reagent blank value from the final results or enter the value as a reagent blank adjust for automatic subtraction.

The graduated cylinder must be completely dry before beginning the test. If the Silver 1 Powder becomes moist, it will not dissolve completely, which will inhibit color development.

The sample pH for this test must be between 9 and 10. Do not use a pH meter to adjust the sample pH as it will contaminate the sample. See *Digestion* for the procedure to adjust pH.

The Pour-Thru Cell cannot be used with this procedure.

Collect the following items:

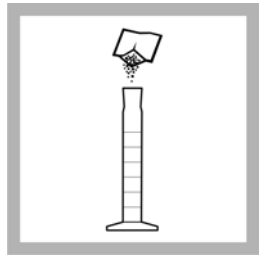
Description	Quantity
Silver 1 Reagent Powder Pillow	1
Silver 2 Reagent Powder Pillow	1
Sodium Thiosulfate Powder Pillow	1
Clippers	1
Cylinder, graduated, 50-mL	1
Cylinder, graduated mixing, 50-mL	1
Sample Cells (see *Instrument-specific information*)	2

See *Consumables and replacement items* for reorder information.

Colorimetric Method

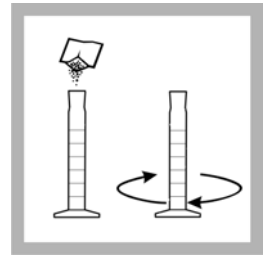
1. Select the test.

Insert an adapter if required (*Instrument-specific information*).



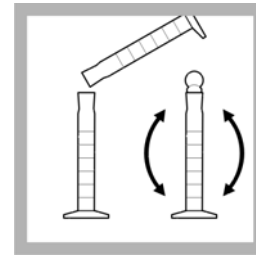
2. Add the contents of one Silver 1 Powder Pillow to a dry 50-mL graduated mixing cylinder.

If the Silver 1 Powder becomes wet at this point, the powder will not dissolve completely, which will inhibit color development.



3. Add the contents of one Silver 2 Reagent Solution Pillow to the cylinder. Swirl to completely wet the powder.

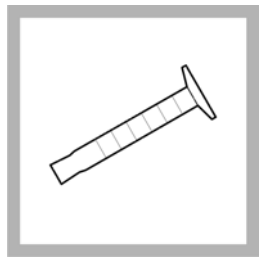
If clumps of dry powder are present when the sample is poured in, the powder will not dissolve completely, which will inhibit color development.



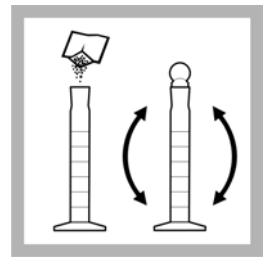
4. Use a 50-mL graduated cylinder to add 50 mL of sample to the 50-mL graduated mixing cylinder. Stopper and invert repeatedly for one minute.

Be sure to first adjust the pH of samples if they were preserved (see *Sample collection, preservation and storage*).

5. Prepared Sample: Fill a sample cell to at least the 10 mL mark with the mixture.



6. Blank Preparation: Discard all but 25 mL of the sample from the mixing cylinder.



7. Add the contents of one Sodium Thiosulfate Reagent Powder Pillow to the sample in the mixing cylinder. Stopper and invert to mix.

Be sure to prepare a blank for each sample.

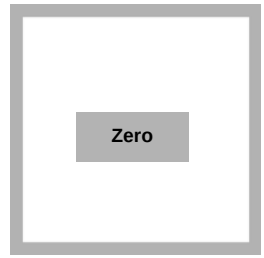
8. Start the instrument timer.

A two-minute reaction time will start.

Colorimetric Method (continued)

9. Fill a second sample cell to at least the 10 mL mark with the mixture.

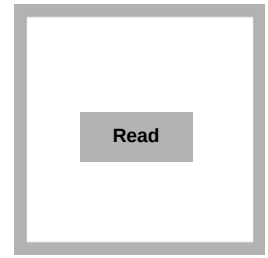
10. When the timer expires, insert the blank in the cell holder.



11. **ZERO** the instrument.

The display will show:

0.00 mg/L Ag



12. Insert the prepared sample in the cell holder.

READ the results in mg/L Ag.

Immediately rinse the cells.

Interferences

Standard solutions of approximately 0.4 mg/L Ag with different concentrations of a potential interfering ion were prepared. The concentration of silver was measured. *Interfering substances* shows the ions that caused a change in the silver concentration of more than ten percent (±10%).

Table 341 Interfering substances

Interfering substance	Interference level
Aluminum	Negative interference above 30 mg/L
Ammonia	Negative interference above 750 mg/L
Cadmium	Negative interference above 15 mg/L
Calcium	Positive interference above 600 mg/L
Chloride	Negative interference above 19 mg/L
Chromium^{6+}	Negative interference above 90 mg/L
Copper	Negative interference above 7 mg/L
Iron	Negative interference above 30 mg/L
Lead	Negative interference above 13 mg/L
Manganese	Negative interference above 19 mg/L
Magnesium	Positive interference above 2000 mg/L
Mercury	Positive interference above 2 mg/L
Nickel	Negative interference above 19 mg/L
Zinc	Negative interference above 70 mg/L

Sample collection, preservation and storage

- Collect samples in acid-cleaned glass or plastic bottles.
- Use pH paper to adjust the pH to 2 or less with concentrated Nitric Acid (about 2 mL/liter).
- Store preserved samples at room temperature for up to 6 months.
- If the sample contains particulates or for a dissolved metal analysis, filter through a 0.45 µm filter at collection. After filtration, adjust the pH to 2 or less for storage.
- Before analysis, adjust the pH to 9–10 with 5.0 N sodium hydroxide. (See steps 11–12 of *Digestion*.)
- Do not use a pH meter because the pH electrode will contaminate the sample.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Silver Standard Solution, 1000 mg/L Ag
- 100-mL Class A volumetric flask
- 5.0 mL Class A volumetric pipet and pipet bulb
- TenSette Pipet, 0.1–1.0 mL and tips

4. After reading test results, leave the sample cell (unspiked sample) in the instrument.
5. Select **OPTIONS>MORE>STANDARD ADDITIONS** from the instrument menu. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
6. Prepare a 50.0 mg/L silver standard solution as follows. Add 5.00 mL of 1000 mg/L Silver Standard Solution to a 100-mL volumetric Class A flask. Dilute to volume with deionized water. This is a 50.0 mg/L standard solution.
7. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of the 50.0 mg/L standard to three 50-mL portions of fresh sample. Mix thoroughly.
8. Follow the *Colorimetric Method* test procedure for each of the spiked samples, starting with the 0.1 mL spiked sample. Measure each of the spiked samples in the instrument.
9. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Required for accuracy check:

- Silver Standard Solution, 1000 mg/L Ag
- 1-L Class A volumetric flask
- 0.5 mL Class A volumetric pipet and pipet bulb

1. Prepare a 0.5 mg/L silver standard solution as follows: Pipet 0.5 mL of silver standard, 1000 mg/L as Ag, into a 1000-mL (1 liter) volumetric flask. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the 0.5-mg/L silver standard solution in place of the sample. Follow the *Colorimetric Method* test procedure.
3. To adjust the calibration curve using the reading obtained with the 0.5-mg/L standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust option and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Digestion

If the sample contains organic matter, thiosulfate or cyanide, digest the sample before analysis. Possible sources for these compounds are wastewater, silver electroplating baths and silver strike solutions. Use the Digesdahl Digestion Apparatus.

DANGER
Chemical hazard. Poisonous hydrogen cyanide gas may be generated during the digestion. Operate the Digesdahl in a closed fume hood.

CAUTION
Chemical hazard. Always wear safety glasses and use a safety shield or operate the Digesdahl in a closed fume hood. Follow the safety precautions in the Digesdahl Digestion Apparatus Manual.

1. Add an appropriate size sample to the 100-mL digestion flask for use with the Digesdahl. Add several boiling chips to prevent bumping.

Note: *Appropriate sample size is determined experimentally. The final sample concentration (after dilution to 100 mL) should be 0–0.6 mg/L. Larger dilutions may be necessary for electroplating baths and silver strike solutions. Do not exceed the maximum sample volume of 25 mL. Several 25-mL aliquots may be digested in succession to concentrate a very dilute sample.*

2. Turn on the water aspirator and make sure there is suction in the fractionating head.
3. Carefully add 3 mL of concentrated sulfuric acid to the sample in the volumetric flask. Immediately place the head on the digestion flask. Never use less than 3 mL of acid.
4. Place the digestion flask on the heater. Turn the temperature dial to 440 °C (825 °F).
5. When the sulfuric acid reflux line becomes visible, wait 3–5 minutes.
6. Make sure there is acid in the flask before adding hydrogen peroxide!
7. Place the capillary funnel on the fractionating head. Fill the funnel to the 10-mL line with 50% hydrogen peroxide.

Note: *If the sample completely evaporates, turn the Digesdahl off and cool completely. Carefully add water to the flask before handling. Start the digestion over with a fresh sample.*

8. Use the capillary funnel to add 5 mL of hydrogen peroxide. Check the solution in the flask for digestion completion. If digestion is not complete, continue adding hydrogen peroxide in 5 mL to 10 mL portions. Several portions may be necessary.

Note: *The digestion is complete when the digestate is colorless or the color of the digestate does not change after hydrogen peroxide is added. A completely digested sample will not cause foam to form.*

9. After digestion is complete and all the hydrogen peroxide has boiled away, reduce the volume of the digestate to near dryness. *Do not allow the sample to become completely dry!* Remove the flask from the heater. Cool to room temperature.

10. Slowly add approximately 25 mL of deionized water to the cooled flask. Swirl to mix.

11. Add 2 drops of 1 g/L Phenolphthalein Indicator Solution. Add 2 drops of 1 g/L Thymolphthalein Indicator Solution.

12. Use sodium hydroxide to adjust the pH of the solution to 9–10. The solution will be pink in this pH range.

Note: *A purple color indicates a pH greater than 10. If this occurs, add a drop of sulfuric acid and 2 drops of each indicator and repeat the pH adjustment. Initially, use 50% sodium hydroxide, then 1 N sodium hydroxide as the end point is approached.*

13. Filter turbid digestates. Quantitatively transfer the filtrate (or unfiltered sample) to a clean 100-mL volumetric flask. Dilute to the mark with deionized water and mix. Follow the *Colorimetric Method* for silver.

Method performance

Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
0.50 mg/L Ag	0.49–0.51 mg/L Ag	0.005 mg/L Ag

Summary of method

Silver ions in basic solution react with cadion 2B to form a green to brown to red-purple complex. The sodium thiosulfate acts as a decolorizing agent for the blank. The Silver 1 and Silver 2 reagents contain the buffer, indicator and masking agents. Organic extractions are not necessary and this method does not have as many interferences as the traditional dithizone method. Test results are measured at 560 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Silver Reagent Set (50 tests), includes:			2296600
Silver 1 Reagent Powder Pillow	1	50/pkg	2293566
Silver 2 Reagent Solution Pillow	1	50/pkg	2293666
Sodium Thiosulfate Powder Pillow	1	50/pkg	2293766

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers	1	each	96800
Cylinder, graduated, 50-mL	1	each	2117941
Cylinder, graduated, mixing, 50-mL	1	each	189641
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Silver Standard Solution, 1000 mg/L Ag	100 mL	1461342
Water, deionized	4 L	27256

Digestion reagents and apparatus

Description	Unit	Catalog number
Hydrogen Peroxide, 50%	490 mL	2119649
Phenolphthalein Indicator Solution, 1 g/L	15 mL SCDB	189736
Sodium Hydroxide Solution, 50%	500 mL	218049
Sodium Hydroxide Solution, 1.00 N	100 mL MDB	104532
Sulfuric Acid, ACS, concentrated	2.5 L	97909
Thymolphthalein Indicator Solution, 1 g/L	15 mL SCDB	2185336
Water, deionized	4 L	27256
Boiling Chips, silicon carbide	500 g	2055734
Digesdahl Digestion Apparatus, 115 VAC, 50/60 Hz	each	2313020
Digesdahl Digestion Apparatus, 230 VAC, 50/60 Hz	each	2313021
Safety Shield, for Digesdahl	each	5003000

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, graduated, 50 mL	each	189641
Nitric Acid, concentrated ACS	500 mL	15249
Sodium Hydroxide, 5.0 N	100 mL	245032
pH paper, 0–14	100/pkg	2601300
Membrane filter paper, 0.45 µm	100/pkg	2618800
Volumetric flask, Class A, 100 mL	each	1457442
Volumetric pipet, Class A, 5 mL	each	1451537
Pipet, TenSette, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Volumetric flask, Class A, 1000 mL	each	1457453
Volumetric pipet, Class A, 0.5 mL	each	1451534
Safety bulb	each	1465100
Finger cots	2/pkg	1464702

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Edition 7

Solids, Settleable Matter

DOC316.53.001202

Direct Measurement[1]

Method 8165

Scope and Application: For sewage and wastewater.

[1] Adapted from Standard Methods for the Examination of Water and Wastewater, Section 2540E.

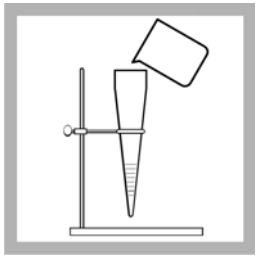
Test preparation

Collect the following items:

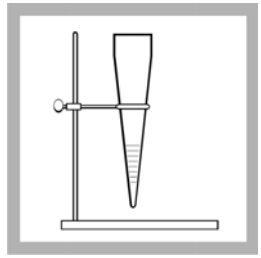
Description	Quantity
Imhoff Cone	2
Imhoff Cone Brush	1
Imhoff Cone Support	1

See *Consumables and replacement items* for reorder information.

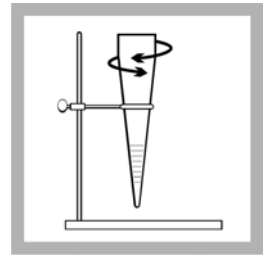
Direct Measurement



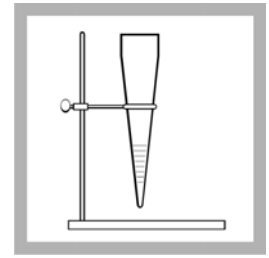
1. Fill an Imhoff cone to the 1-liter mark with a thoroughly mixed sample.



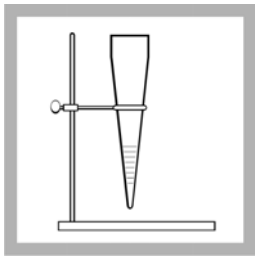
2. Wait 45 minutes for the undisturbed sample to settle.



3. Spin the cone forward and backward several times to dislodge materials on the inclined side of the cone.



4. Wait 15 minutes for the sample to settle.



5. Read the graduated scale on the Imhoff cone at the top of the solids layer as mL/L settleable matter.

Sampling and storage

- Collect samples in clean plastic or glass bottles.
- Refrigerate at 4 °C up to the time of analysis to minimize microbiological decomposition of solids.
- Analyze within 24 hours. Bring to room temperature before analysis.

Summary of method

The amount of settleable matter in sewage treatment plant influent and effluent gives an empirical estimate of the type and extent of treatment required and the general quality of the water being discharged.

Consumables and replacement items

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Imhoff Cone	2	each	206700
Imhoff Cone Brush	1	each	68800
Imhoff Cone Support	1	each	57200

Optional apparatus

Description	Quantity/Test	Unit	Catalog number
Beaker, Glass, low form	2000 mL	each	50054
Gloves, Chemical resistant, size 9–9 1/2	pair	each	2410104
Pitcher, Graduated	2000 mL	each	2612854
Sampler, Dipper	3–9 ft handle	each	2929501

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Edition 7

Solids, Nonfilterable Suspended Solids; Total and Volatile

DOC316.53.001204

USEPA[1] Gravimetric Method[2] — Methods 8158 and 8164

Scope and Application: For water and wastewater.

[1] USEPA accepted.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater* Section 2450

Test preparation

Before starting the test:

The Total Nonfilterable Solids are the same as the Total Suspended Solids (TSS)

Collect the following items:

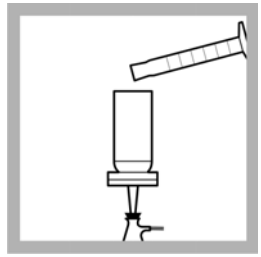
Description	Quantity
Filter flask	1
Filter holder	1
Filter, 47-mm	1
Graduated cylinder, 100-mL	1
Tongs	1
Tweezers	1
Watch glass	1
Desiccator with desiccant	1
Muffle Furnace	1
Drying Oven	1
Deionized Water	varies

See *Consumables and replacement items* for reorder information.

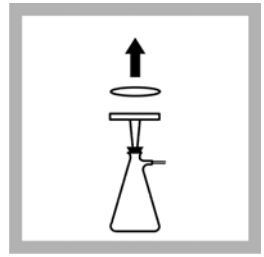
Gravimetric Method—Total Nonfilterable Residue, Method 8158

1. Use tweezers to place a 47-mm glass fibre filter disc in the filter holder. Always use tweezers to handle filter discs.

Moisture from fingers can add moisture to the disc and cause a weighing error.

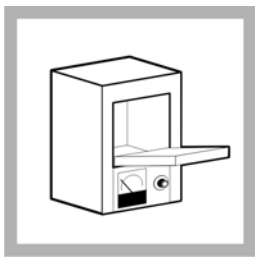


2. Place the filter holder assembly in the filtering flask and add 100 mL of deionized water. Apply vacuum to the flask until all the water is drawn through the filter.



3. Slowly release the vacuum from the filtering system and remove the disc from the filter holder and transfer to a watch glass.

4. Place the disc in a preheated drying oven at 103 °C for one hour.

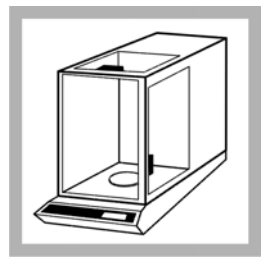


5. If volatile nonfilterable solids are also being measured, use tongs to place the watch glass with the disc into a muffle furnace and ignite at 550 °C for 15 minutes. If not, omit this step.

Partially preheat the muffle furnace before inserting the watch glass. Placing the watch glass in a 550 °C furnace could cause it to shatter. Bring the temperature up to 550 °C 15 minutes after placing the filter and watch glass in the furnace.

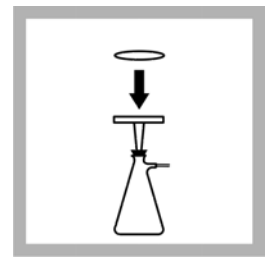
6. Use metal tongs to remove the disc and watch glass from the oven or furnace and place in a desiccator. Cover immediately. Allow the watch glass to cool slightly before sealing the desiccator as pressure from the heated air inside the desiccator can force the cover off.

Allow the filter and glass to cool to room temperature.



7. Remove the watch glass and disc from the desiccator as a unit and place beside the analytical balance.

Use plastic tweezers to remove the disc from the watch glass and weigh to the nearest 0.1 mg (0.0001 g). Record this value as B.



8. Again, place the disc in the filter holder/flask assembly. Wet the disc with deionized water to ensure adhesion to the holder.

Gravimetric Method—Total Nonfilterable Residue, Method 8158 (continued)

9. Filter 100 mL (or more, if solids content is low) of well-mixed, representative water sample by applying vacuum to the flask. Follow with three separate 10-mL washings of deionized water.

For greatest accuracy, filter as much sample as possible. However, using a sample that contains more than 15 mg of solids will clog the filter prematurely. Adjust the exact volume of the water sample to achieve the optimum condition. Several completed tests will show whether any adjustment is necessary.

10. Remove any residue that remains on the sides or bottom lip of the filter holder. A rubber policeman on the end of a stirring rod is very helpful to scrape the residue loose. Small amounts of deionized water will help wash the residue down onto the filter disc.

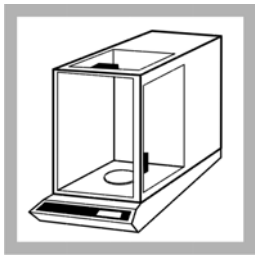
Slowly release the vacuum from the filtering system and gently remove the filter disc from the holder. Place the disc on a watch glass. Inspect the filtrate (filtered water in flask) to make sure that the solids are properly trapped on the disc.

11. Place the watch glass and filter in a drying oven at 103 °C for one hour.

12. Use metal tongs to remove the disc and watch glass from the oven or furnace and place in a desiccator. Cover immediately. Allow the watch glass to cool slightly before sealing the desiccator as pressure from the heated air inside the desiccator can force the cover off.

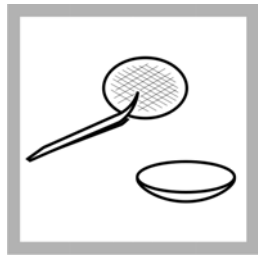
Allow the filter and glass to cool to room temperature.

Gravimetric Method—Total Nonfilterable Residue, Method 8158 (continued)



13. Remove the watch glass and disc from the desiccator as a unit and place beside the analytical balance.

Use plastic tweezers to remove the disc from the watch glass and weigh to the nearest 0.1 mg (0.0001 g). Record this mg value as A.



14. Return the disc to the watch glass if the mg/L Volatile Nonfilterable Residue is to be determined. If not, discard the disc.

If Volatile Nonfilterable Residue is to be determined, do not lose any of the suspended matter on the disc.

15. Calculate Total Non-filterable Residue (TNR):

$$\frac{A - B}{\text{Sample Volume in Liters}} = \text{mg/L TNR}$$

Where:

A = Weight (mg) of disc with residue

B = Weight (mg) of disc

Example:

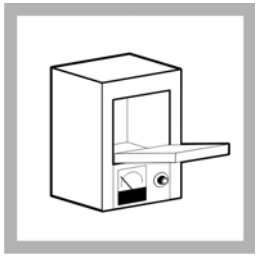
A = 95.5 mg

B = 81.5 mg

Volume of sample = 0.1 L

$$\frac{95.5\text{ mg} - 81.5\text{ mg}}{0.1\text{ L}} = 140\text{ mg/L TNR}$$

Gravimetric Method—Volatile Nonfilterable Solids, Method 8164

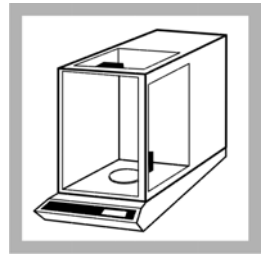


1. Place the watch glass and filter disc from the Total Nonfilterable Residue procedure (step 14) in the muffle furnace and ignite at 550 °C for 15 minutes.

Partially preheat the muffle furnace before inserting the watch glass. However, placing the watch glass in a 550 °C furnace could cause it to shatter. Bring the temperature up to 550 °C 15 minutes after placing the filter and watch glass in the furnace.

2. Use metal tongs to remove the disc and watch glass from the oven or furnace and place in a desiccator. Cover immediately. Allow the watch glass to cool slightly before sealing the desiccator as pressure from the heated air inside the desiccator can force the cover off.

Allow the filter and glass to cool to room temperature.



3. Remove the watch glass and disc from the desiccator as a unit and place beside the analytical balance.

Use plastic tweezers to remove the disc from the watch glass and weigh to the nearest 0.1 mg (0.0001 g). Record this mg value as C.

4. Calculate Volatile Non-filterable Residue (VNR):

$$\frac{A - C}{\text{Sample Volume in Liters}} = \text{mg/L VNR}$$

where:

A = Weight (mg) of disc with residue

C = Weight (mg) of disc and residue after ignition

Example:

A = 95.5 mg

C = 91.2 mg

Volume of sample = 0.1 L

$$\frac{95.5\text{ mg} - 91.2\text{ mg}}{0.1\text{ L}} = 43\text{ mg/L VNR}$$

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Samples should be analyzed as soon as possible after collection but can be stored up to seven days by cooling to 4 °C (39 °F).

Consumables and replacement items

Required apparatus

Description	Unit	Catalog number
Aspirator, vacuum	each	213100
Balance, Analytical, 115 VAC, 60 Hz	each	2936701
Bottle, wash, 500-mL	each	62011
Cylinder, graduated, 100-mL	each	50842
Desiccant, indicating Drierite	each	2088701
Desiccator, without stopcock	each	1428500
Desiccator Plate, ceramic	each	1428400
Filter disc, glass fiber, 47-mm	100/pkg	253000
Filter Holder, magnetic	each	1352900
Flask, filtering, 1000-mL	each	54653
Furnace, muffle 240 VAC, 50/60 Hz	each	1429624
Furnace, muffle, 120 VAC, 50/60 Hz	each	1429600
Oven, laboratory, 240 VAC, 50 Hz	each	1428902
Oven, laboratory, 120 VAC, 60 Hz	each	1428900
Stopper, rubber, one-hole, No. 8	6/pkg	211908
Tongs	each	56900
Tubing, rubber, 7.9 x 2.4 mm	3.6 m	56019
Tweezers, plastic	each	1428200
Watch Glass, 100-mm	each	57870
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, approx.. 58% ACS	500 mL	10649
Bottle, w/cap, wide mouth 500 mL poly	12/pkg	2087079
Brush	each	68700
Pump, vacuum, hand-operated	each	1428300
Pump, vacuum, 1.2 CFM, 220 VAC, w/Europeon plug	each	—
Pump, vacuum, 1.2 CFM, 115 VAC, 60 Hz	each	2824800
Rubber policeman for 3/16" rod	each	1430900
Stirring rod, glass	3/pkg	177001

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Edition 7

Solids, Total Filterable (Total Dissolved Solids)

DOC316.53.001246

USEPA[1] Gravimetric Method[2]

Method 8163

Scope and Application: For water and wastewater.

[1] USEPA accepted.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater, Part 2540C*

Test preparation

Before starting the test:

Total Filterable Solids = Total Dissolved Solids (TDS)
Samples with high bicarbonate concentrations may require increased drying at 180 °C to make sure that the conversion of bicarbonate to carbonate is complete.
Limit sample size to no more than 200 mg residue for best results.
When measuring volatile dissolved solids, heat the evaporating dish to 550 °C for 1 hour before beginning the test.
Sample residue from this procedure can be used directly in Method 8277, Solids, Total Volatile and Fixed.

Collect the following items:

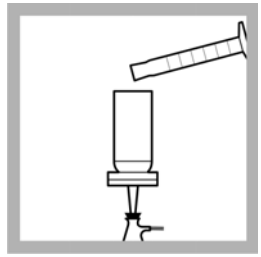
Description	Quantity
Evaporating dish	1
Filter flask	1
Filter holder	1
Filter, 47-mm	1
Graduated cylinder, 100-mL	1
Hot Plate	1
Steam Bath, 8" diameter	1
Analytical Balance	1
Desiccator	1
Tongs	1
Tweezers	1
Drying Oven	1
Deionized Water	varies

See *Consumables and replacement items* for reorder information.

USEPA Gravimetric Method—Total Filterable Solids, Method 8163

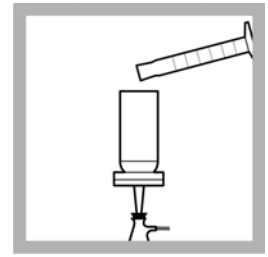
5. Heat a clean evaporating dish in a drying oven at 180 °C for one hour.

Using metal tongs, remove the evaporating dish from the furnace and place in a desiccator. Cover immediately. Allow the dish to cool slightly before sealing the desiccator as pressure from the heated air inside the desiccator can force the cover off.



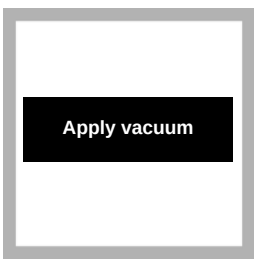
6. Assemble the filter holder/flask assembly using a clean filter flask. Before using the flask, remove all residue from the flask by thoroughly cleaning with a dilute solution of ammonium hydroxide and rinsing with deionized water.

7. Place a 47-mm filter disc in the filter holder. With vacuum applied to the flask, wash the filter with three separate 20-mL volumes of deionized water. Remove all traces of water by continuing vacuum for two to three minutes after the water has passed through the filter. Disconnect the vacuum and discard these washings from the flask.

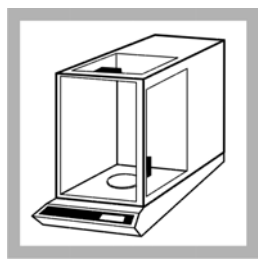


8. Reconnect vacuum to the filter holder/flask assembly. Use a clean 100-mL graduated cylinder to filter 100 mL (or more if solids content is low) of a well-mixed representative water sample.

For greatest accuracy, filter as much sample as possible. However, using a sample that contains more than 15 mg of solids will clog the filter prematurely. Adjust the exact volume of the water sample to achieve the optimum condition. Several completed tests will show whether any adjustment is necessary.

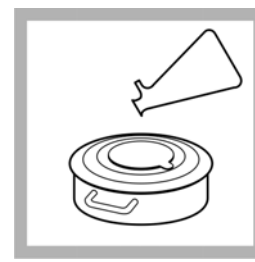


9. Apply vacuum for two to three minutes after the sample has passed through the filter. Disconnect the vacuum.



10. Use tongs to transfer the evaporating dish from the desiccator to the balance. Weigh to the nearest 0.1 mg (0.0001 g) and record this weight as B.

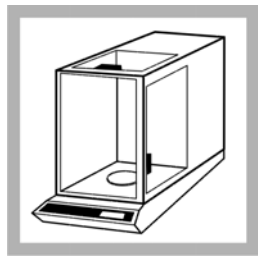
11. Place the steam bath on the hot plate, add water and transfer the evaporating dish from the balance to the steam bath.

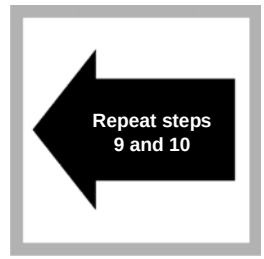


12. Pour the 100-mL filtrate sample from the filter flask into the evaporating dish. Evaporate to dryness (this may take up to four hours).

Periodically check the reservoir of the water bath. Add more water if needed.

USEPA Gravimetric Method—Total Filterable Solids, Method 8163 (continued)





13. Use tongs to transfer the evaporating dish residue to a drying oven and dry at 180 °C for one hour. Transfer to a desiccator and cool.

Allow the dish to cool slightly before sealing the desiccator; pressure from the heated air inside the desiccator can force the cover off.

14. Weigh the evaporating dish to the nearest 0.1 mg (0.0001 g) on an analytical balance and record this weight as A.

15. Repeat steps *13* and 14 until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less.

The solid residue can be used directly in method 8277 to determine Volatile Dissolved Solids.

16. Calculate the Total Filterable Residue (TFR). Refer to *Total filterable residue calculation*.

Total filterable residue calculation

To calculate TFR:

$$\frac{A - B}{\text{Sample volume in liters}} = \text{mg/L TFR}$$

Where:

A = Weight (mg) of residue and dish after drying

B = Weight (mg) of dish Sample Volume = 0.1 L

Example:

A = 20187.3 mg

B = 20140.1 mg

$$\frac{20187.3 - 20140.1}{0.1} = 472 \text{ mg/L TFR}$$

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Samples should be analyzed as soon as possible after collection but can be stored up to seven days by cooling to 4 °C (39 °F).

Summary of method

A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated to dryness in a weighed dish and dried to a constant weight at 180 °C. The increase in the weight of the dish after drying represents the total filterable solids (total dissolved solids).

Consumables and replacement items

Required apparatus

Description	Unit	Catalog number
Aspirator, vacuum	each	213100
Balance, Analytical, 115 VAC, 60 Hz	each	2936701
Bottle, wash, 500-mL	each	62011
Cylinder, graduated, 100-mL	each	50842
Desiccant, indicating Drierite	each	2088701
Desiccator, without stopcock	each	1428500
Desiccator Plate, ceramic	each	1428400
Evaporating dish, porcelain, w/lip, 120-mL, 90-mm		
Filter disc, glass fiber, 47-mm	100/pkg	253000
Filter Holder, magnetic	each	1352900
Flask, filtering, 1000-mL	each	54653
Hot plate/stirrer, 7 x 7 inch, 115 VAC	each	2881600
Hot plate/stirrer, 7 x 7 inch, 220–240 VAC	each	2881602
Oven, laboratory, 240 VAC, 50 Hz	each	1428902
Oven, laboratory, 240 VAC, 50 Hz	each	1428902
Oven, laboratory, 120 VAC, 60 Hz	each	1428900
Steam bath, 8-inch diameter		2347900
Stopper, rubber, one-hole, No. 8	6/pkg	211908
Tongs	each	56900
Tubing, rubber, 7.9 x 2.4 mm	3.6 m	56019
Tweezers, plastic	each	1428200
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Ammonium Hydroxide, 58% ACS	500 mL	10649
Bottle, w/cap, wide mouth 500 mL poly	12/pkg	2087079
Brush	each	68700
Pump, vacuum, hand-operated	each	1428300
Pump, vacuum, 1.2 CFM, 220 VAC, w/Europeon plug	each	—
Pump, vacuum, 1.2 CFM, 115 VAC, 60 Hz	each	2824800
Rubber policeman for 3/16" rod	each	1430900
Stirring rod, glass	3/pkg	177001

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Edition 7

Solids, Volatile Dissolved and Fixed Dissolved

DOC316.53.001206

Gravimetric Method[1]

Method 8277

Scope and Application: For wastewater.

[1] Adapted from Standard Methods for the Examination of Water and Wastewater

Test preparation

Before starting the test:

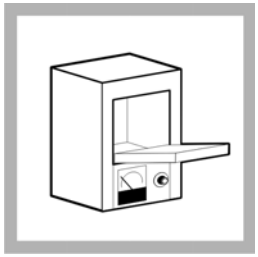
The residue from Method 8163—Solids, Total Filterable can be used in this method. Start at Step 12..

Collect the following items:

Description	Quantity
Filter flask	1
Filter holder assembly with stopper	1
Filter, 47-mm, glass fiber	1
Graduated cylinder, 100-mL	1
Tongs	1
Evaporating dish	1
Desiccator with desiccant	1
Analytical balance	1
Muffle furnace	1
Vacuum sourceand tubing	1
Deionized water	1
Steam bath	1
Hot plate	1

See *Sample collection, preservation and storage* for reorder information.

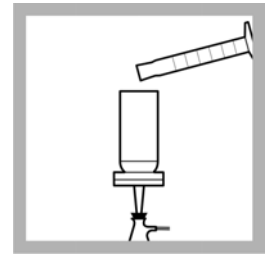
Gravimetric Method



1. Heat an evaporating dish at 550 °C for one hour. Cool and store the dish in a desiccator until needed. Allow the dish to cool slightly before sealing the desiccator, as pressure from the heated air can force the cover off.

2. Assemble the filter holder/flask assembly using a clean filter flask. All residue should be removed from the flask by cleansing thoroughly with a dilute solution of ammonium hydroxide and rinsing with deionized water.

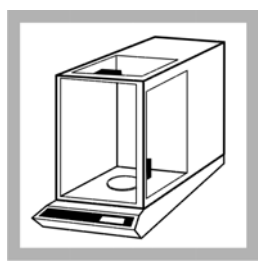
3. Place a 47-mm filter disc in the filter holder. With vacuum applied to the flask, wash the filter with three separate 20-mL volumes of deionized water. Remove all traces of water by continuing vacuum for two to three minutes after the water has passed through the filter. Disconnect the vacuum and discard these washings from the flask.



4. Reconnect vacuum to the filter holder/flask assembly. Using a clean 100-mL graduated cylinder, filter 100 mL (or more if solids content is low) of a well-mixed representative water sample.

For greatest accuracy, filter as much sample as possible. However, using a sample that contains more than 15 mg of solids will clog the filter prematurely. Adjust the exact volume of the water sample to achieve the optimum condition. Several completed tests will show whether any adjustment is necessary.

5. Apply vacuum for two to three minutes after the sample has passed through the filter. Disconnect the vacuum.



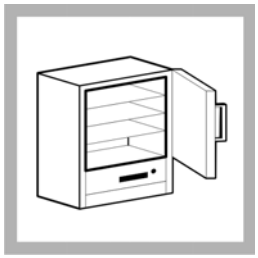
6. Use tongs to transfer the evaporating dish from the desiccator to the balance. Weigh to the nearest 0.1 mg (0.0001 g) and record this weight as A.

7. Place the steam bath on the hot plate, add water and transfer the evaporating dish from the balance to the steam bath.

8. Pour the 100-mL filtrate sample from the filter flask into the evaporating dish. Evaporate to dryness (this may take up to four hours).

Periodically check the reservoir of the water bath. Add more water if needed.

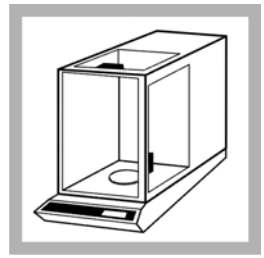
Gravimetric Method (continued)



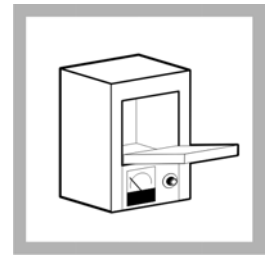
9. Place the dish in a pre-heated oven at 103-105 °C and dry to a constant weight. This will take 30–60 minutes.

10. Take the dish out of the oven and allow it to cool to room temperature in a desiccator.

Allow the dish to cool slightly in the desiccator before sealing the desiccator, as pressure from the heated air can force the cover off.

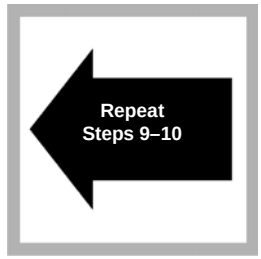


11. Use tongs to transfer the evaporating dish to the balance. Weigh to the nearest 0.1 mg (0.0001 g). Record this as B.



12. Transfer the dish into a preheated muffle furnace at 550 °C for 30 minutes.

13. Take the dish out of the furnace with tongs and cool to room temperature in a desiccator. Weigh the dish to the nearest 0.1 mg (0.0001 g) using an analytical balance. Record this mg value as D.



14. Repeat the ignition (steps 12. and 13.) until two successive weighings do not differ by more than 4% or 0.5 mg, whichever is greater.

15. The loss of weight is total volatile solids. Weighed residue is total fixed solids.

Calculate:

mg/L Volatile Diss. Solids =

$$\frac{(B - D) \times 1000}{C}$$

mg/L Fixed Diss. Solids =

$$\frac{(A - D) \times 1000}{C}$$

Where:

B = Weight (mg) of solids + dish before ignition

D = Weight (mg) of solids + dish after ignition

C = Volume in mL of filtrate transferred to the aluminum dish

A = Weight (mg) of dish

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Samples should be analyzed as soon as possible after collection, but can be stored up to seven days by cooling to 4 °C (39 °F).

Summary of method

A well-mixed sample is filtered through a glass fiber filter. An aliquot of the filtrate is evaporated in a weighed dish and dried to constant weight in a 103–105 °C oven. The dish and sample residue are ignited at 550 °C for 30 minutes. The loss of sample mass upon ignition represents the volatile dissolved solids. The remaining residue after ignition represents the fixed dissolved solids.

Consumables and replacement items

Required apparatus

Description	Unit	Catalog number
Balance, Analytical, SA80, 115 VAC, 60 Hz	each	2936701
Cylinder, graduated, 100-mL	each	50842
Desiccant, indicating Drierite	each	2088701
Desiccator, without stopcock	each	1428500
Desiccator Plate, ceramic	each	1428400
Evaporating dish, porcelain, w/lip, 120-mL, 90-mm	each	52561
Furnace, muffle 120 VAC 50/60 Hz	each	1429600
Furnace, muffle 240 VAC 50/60 Hz	each	1429624
Steam bath, 8” diameter	each	2347900
Tongs	each	56900
Aspirator, vacuum	each	213100
Filter disc, 47 mm, glass fiber	100/pkg	253000
Filter Holder, 47 mm magnetic	each	1352900
Flask, filtering 1000 mL	each	54653
Hot plate/stirrer, 7 x 7 inch, 115 VAC	each	2881600
Hot plate/stirrer, 7 x 7 inch, 220-240 VAC	each	2881602
Oven, laboratory, 120 VAC, 60 Hz	each	1428900
Oven, laboratory, 240 VAC, 50 Hz	each	1428902
Stopper, rubber, one-hole No. 8	6/pkg	211908
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Dish, aluminum (63 x 17.5 mm)	100/pkg	2164000
Ammonium Hydroxide, 10%	500 mL	1473649
Blender,115 VAC, 1.2 L	each	2616100
Blender, 240 VAC, 1.2 L	each	2616102
Bottles, w/cap, wide mouth, 500 mL poly	12/pkg	2087079
Beaker, glass, 250 mL	each	50046H
Pump, Vacuum, 1.2 cfm, 115 VAC	each	2824800
Pump, Vacuum, 1.2 cfm, 220 VAC, European plug	each	2824802
Pump, Vacuum, Hand-operated	each	1428300
Stir Bar, 22 x 8 mm	each	2095350
Stirrer, magnetic, 4 x 4, 115 VAC	each	2881200
Stirrer, magnetic, 4 x 4, 230 VAC	each	2881202

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Edition 7

Solids, Total Volatile and Fixed

DOC316.53.001205

Gravimetric Method[1]

Method 8276

Scope and Application: For potable, surface and saline water and domestic and industrial wastewater.

[1] Adapted from Standard Methods for the Examination of Water and Wastewater.

Test preparation

Before starting the test:

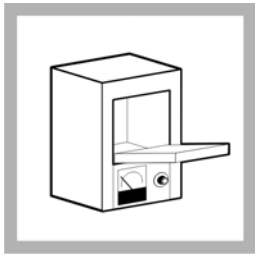
When measuring volatile solids, ignite the aluminum dishes for one hour at 550 °C prior to use.
Larger sample sizes may be used. For larger samples, use an evaporating dish and a steam bath to evaporate the liquid.
Samples from Method 8271 may be used directly in this procedure. Use the resulting sample and start this method at step 7.

Collect the following items:

Description	Quantity
Weighing dish, aluminum	1
Drying oven	1
Graduated cylinder, 50 mL	1
Desiccator and desiccant	1
Analytical balance	1
Muffle furnace	1

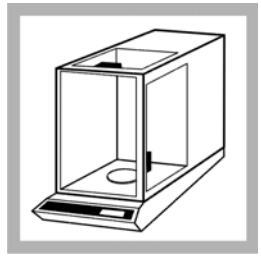
See *Consumables and replacement items* for reorder information.

Gravimetric Method

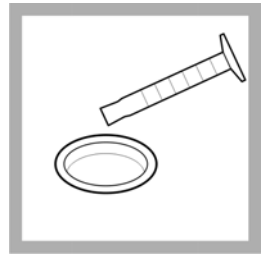


1. Heat an aluminum dish to 550 °C for one hour. Store and cool the dish in a desiccator until needed.

If a sample from Method 8271 is used, omit steps 2–6. Proceed to step 7.



1. Weigh an aluminum dish to the nearest 0.1 mg (0.0001 g). Record this mg value as C.



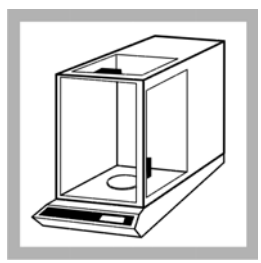
2. Mix the sample and add 50 mL to the aluminum dish. Use a blender or a beaker with stir bar and stir plate to mix the samples.

3. Place the sample in a preheated oven and evaporate at 103–105 °C for approximately six hours.

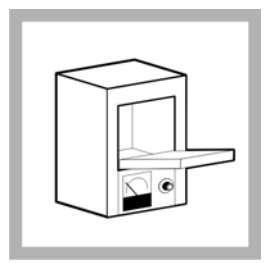
Highly mineralized water may prolong the drying time.

Note: *A steam bath and evaporating dish may be used in place of the drying oven for larger samples. After evaporation on the steam bath, dry the dish to constant weight in a 103–105 °C drying oven.*

4. Take the dish out of the oven and allow it to cool to room temperature in a desiccator.

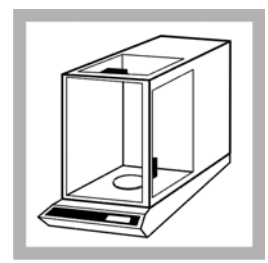


5. Weigh the dish to the nearest 0.1 mg (0.0001 g) using an analytical balance. Record this mg value as A.



6. Transfer the aluminum dish into a pre-heated muffle furnace at 550 °C for 30 minutes.

After 30 minutes, take the dish out of the furnace with tongs and cool to room temperature in a desiccator.



7. Weigh the dish to the nearest 0.1 mg using an analytical balance.

Repeat ignition until two successive sample weighings (A – B) do not differ by more than 4% or 0.5 mg, whichever is less. Record this mg value as B.

Gravimetric Method (continued)

8. The loss of weight is total volatile solids. Weighed residue is total fixed solids.

Calculate:

mg/L Volatile Solids

$$= \frac{(A - B) \times 1000}{\text{sample in volume in mL}}$$

mg/L Fixed Solids

$$= \frac{(B - C) \times 1000}{\text{sample volume in mL}}$$

where:

A = Weight (mg) of solids + dish before ignition at 550 °C

B = Weight (mg) of solids + dish after ignition at 550 °C

C = Weight (mg) of empty dish

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Samples should be analyzed as soon as possible after collection, but can be stored up to seven days by cooling to 4 °C (39 °F).

Summary of method

A well-mixed sample is evaporated in a weighed dish and dried to a constant weight in a 103–105 °C oven. The dish and sample are ignited at 550 °C for 30 minutes. The loss of sample mass upon ignition represents the volatile solids. The remaining sample after ignition represents the fixed solids.

Consumables and replacement items

Required apparatus

Description	Unit	Catalog number
Balance, Analytical, 115 VAC, 60 Hz	each	2936701
Cylinder, graduated, 50 mL	each	50841
Desiccant, indicating Drierite	each	2088701
Desiccator, without stopcock	each	1428500
Desiccator Plate, ceramic	each	1428400
Dish, aluminum (63 x 17.5 mm)	100/pkg	2164000
Furnace, muffle, 120 Vac, 50/60 Hz	each	1429600
Furnace, muffle, 240 Vac, 50/60 Hz	each	1429624
Oven, laboratory, 240 Vac, 50 Hz	each	1428902
Water, deionized	4 L	27256
Oven, laboratory, 120 VAC, 60 Hz	each	1428900
Tongs	each	56900

Optional reagents and apparatus

Description	Unit	Catalog number
Blender, 1.2 liter, 120 VAC	each	2616100
Blender, 1.2 liter, 240 VAC	each	2616102
Stirrer, Magnetic, 4 x 4, 115 VAC	each	2881200
Stirrer, magnetic, 4 x 4, 230 VAC	each	2881202
Beaker, Glass, 250 mL	each	50046H
Stir Bar, 22 x 8 mm	each	2095350
Steam Bath, 8 inch diameter	each	2347900
Evaporating Dish, porcelain, 120 mL	each	52561
Bottles, w/cap, wide mouth, 500 mL poly	12/pkg	2087079

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Edition 7

Solids, Total

DOC316.53.001203

USEPA[1] Gravimetric Method[2]

Method 8271

Scope and Application: For potable, surface and saline water and for domestic and industrial wastewater.

[1] USEPA Accepted.

[2] Adapted from Standard Methods for the Examination of Water and Wastewater, Section 2540B

Test preparation

Before starting the test:

Test results may be used directly in continuation with Method 8276—Solids, Total Volatile and Fixed
Dry the aluminum dishes at 103-105C for one hour prior to use. Store dried dishes in a desiccator.
Larger samples can be used. For larger samples, use a steam bath and evaporating dishes in place of the aluminum dishes.

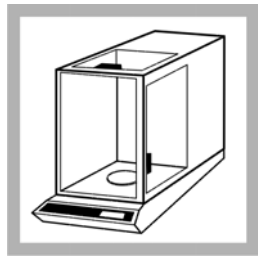
Collect the following items:

Description	Quantity
Weighing dish, aluminum	1
Drying oven	1
Graduated cylinder, 50 mL	1
Desiccator with desiccant	1
Analytical balance	1
Tongs	1

See *Sample collection and storage* for reorder information.

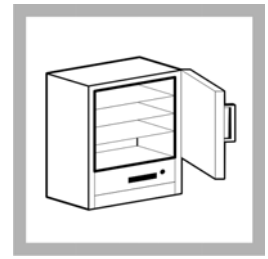
Gravimetric Method

1. Heat an aluminum dish at 103–105 °C for one hour. Store and cool the dish in a dessicator.



2. Weigh the aluminum dish to the nearest 0.1 mg (0.0001 g). Record the mg value as B.

3. Mix the sample and add 50 mL to the aluminum dish. Use a blender or a beaker with stirbar and stir plate to mix samples.

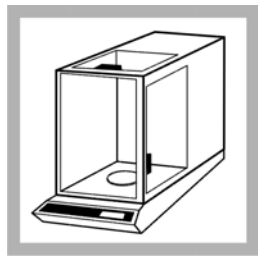


4. Place the sample in a preheated oven and evaporate at 103–105 °C for approximately six hours.

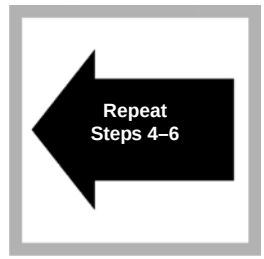
Highly mineralized water may prolong the drying time.

Note: *A steam bath and evaporating dish may be used in place of the drying oven for larger samples. After evaporating on the steam bath, dry the dish to constant weight in a 103-105C drying oven.*

5. Take the dish out of the oven and allow it to cool to room temperature in a desiccator.



6. Weigh the dish to the nearest 0.1 mg (0.0001 g) using an analytical balance. Record this mg value as A.



7. Repeat step 3–step 5 until results do not differ by more than 0.4 mg.

Successive weighings that are identical for some wastewater samples are unlikely due to slow organic volatilization.

Gravimetric Method (continued)

8. Calculate:

$$\text{mg/L Total Solids} = \frac{(A - B) \times 1000}{\text{Sample Volume in mL}}$$

Where:

A = Weight (mg)[1] of sample + dish

B = Weight (mg) of dish

Note: *Continue with Method 8276 if Volatile and Fixed Solids results are required.*

[1] Weight in mg = grams x 1000

Sample collection and storage

- Collect samples in clean plastic or glass bottles.
- Samples should be analyzed as soon as possible after collection, but can be stored up to seven days by cooling to 4 °C (39 °F).

Summary of method

A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 102–105 °C. The increase in weight over that of the empty dish represents the total solids.

Consumables and replacement items

Required apparatus

Description	Unit	Catalog number
Balance, Analytical, 115 VAC, 60 Hz	each	2936701
Cylinder, 50-mL	each	50841
Desiccant, indicating Drierite	each	2088701
Desiccator, without stopcock	each	1428500
Desiccator Plate, ceramic	each	1428400
Water, deionized	4 L	27256
Dish, aluminum (63 x 17.5 mm)	100/pkg	2164000
Oven, laboratory, 120 VAC, 60 Hz	each	1428900
Tongs	each	56900
Oven, laboratory, 240 VAC, 50 Hz	each	1428902

Optional reagents and apparatus

Description	Unit	Catalog number
Blender, 1.2 liter, 120 VAC	each	2616100
Blender, 1.2 liter, 240 VAC	each	2616102
Stirrer, Magnetic, 4 x 4, 115 VAC	each	2881200
Stirrer, magnetic, 4 x 4, 230 VAC	each	2881202
Beaker, Glass, 250 mL	each	50046H
Stir Bar, 22 x 8 mm	each	2095350
Steam Bath, 8 inch diameter	each	2347900
Evaporating Dish, porcelain, 120 mL	each	52561
Bottles, w/cap, wide mouth, 500 mL poly	12/pkg	2087079

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Edition 7

Suspended Solids

DOC316.53.01139

Photometric Method[1] **Method 8006**

(5 to 750 mg/L)

Scope and Application: For water and wastewater.

[1] *Adapted from Sewage and Industrial Wastes, 31, 1159 (1959).*

Test preparation

How to use instrument-specific information

The*Instrument-specific information* table displays information that may vary from instrument to instrument. Select your spectrophotometer from the instrument column on the left. Read across to find the corresponding sample cells and adapters required to perform this test on your spectrophotometer.

Table 342 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

The Pour-Thru Cell cannot be used with this procedure.

Collect the following items:

Description	Quantity
Beaker, 600-mL, polypropylene	1
Blender	1
Cylinder, 500-mL polypropylene, graduated	1
Sample Cells (see the *Instrument-specific information* table)	2

See *Consumables and replacement items* for reorder information.

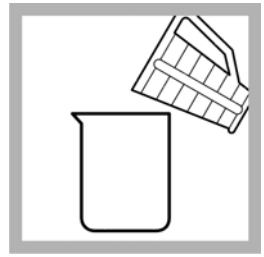
Photometric Method

1. Select the test.

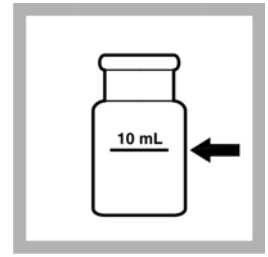
Insert an adapter if required (see the *Instrument-specific information* table).

Refer to the user manual for orientation.

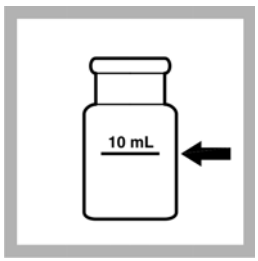
2. Blend 500 mL of sample in a blender at high speed for exactly two minutes.



3. Pour the blended sample into a 600-mL beaker.

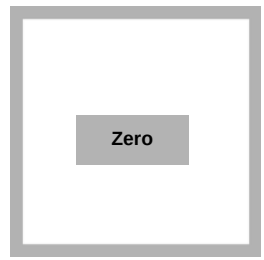


4. Prepared Sample: Stir the sample and immediately pour 10 mL of the blended sample into a sample cell.



5. Blank Preparation: Fill a second sample cell with 10 mL of tap water or deionized water.

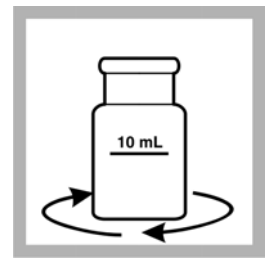
6. Wipe and insert the blank into the cell holder.



7. ZERO the instrument.

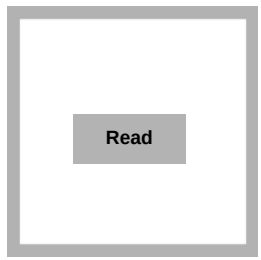
The display will show:

0 mg/L TSS



8. Swirl the prepared sample to remove any gas bubbles and uniformly suspend any residue.

9. Wipe and insert the prepared sample into the cell holder.



10. READ the results in mg/L TSS.

Interferences

Samples that absorb strongly at 810 nm, such as blue dyes, may give false, high-bias readings. A user-entered calibration is advised for these samples.

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Analyze samples as soon as possible after collection. The sample may be stored for seven days by cooling to 4 °C (39 °F).

Accuracy check

Calibration for this test is based on parallel samples using the gravimetric technique on sewage samples from a municipal sewage plant. For most samples, this calibration will provide satisfactory results. When higher accuracy is required, run parallel spectrophotometric and gravimetric determinations with portions of the same sample. Make the new calibration on the particular sample using a gravimetric technique as a basis.

Summary of method

This method of determining suspended solids is a simple, direct measurement which does not require the filtration or ignition/weighing steps that gravimetric procedures do. The USEPA specifies the gravimetric method for solids determinations, while this method is often used for checking in-plant processes. Test results as mg/L total suspended solids (TSS) are measured at 810 nm.

Consumables and replacement items

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 600-mL, polypropylene	1	each	108052
Blender, 1.2-L, 120 VAC	1	each	2616100
Blender, 1.2 L, 240 VAC	1	each	2616102
Cylinder, 500-mL graduated, polypropylene	1	each	108149

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Edition 7

Sulfate

DOC316.53.01135

USEPA[1] SulfaVer 4 Method[2] **Method 8051**

(2 to 70 mg/L) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater.

[1] USEPA accepted for reporting wastewater analyses. Procedure is equivalent to USEPA method 375.4 for wastewater.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 343 Instrument-specific information

Instrument	Powder pillows		AccuVac Ampuls	
	Sample cell	Cell orientation	Sample cell	Adapter
DR 6000	2495402	Fill line faces right	2427606	—
DR 5000	2495402	Fill line faces user	2427606	—
DR 3900	2495402	Fill line faces user	2427606	LZV846 (A)
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right	2122800	LZV584 (C)

Before starting the test:

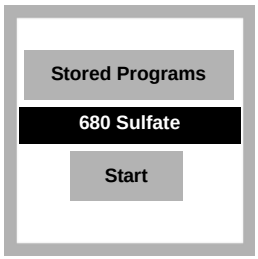
Adjust the standard curve for each new lot of reagent (*Standard solution method*).
For best results, calibrate the instrument with each new lot of reagent (see *Calibration*).
For best results, measure a reagent blank value for each new lot of reagent (follow the procedure using deionized water in place of the sample). Subtract the reagent blank value from the final results or enter the value as a reagent blank adjust for automatic subtraction.
Filter highly colored or turbid samples using filter paper and a funnel. Use this sample in step 2 and 5.
The Pour-Thru Cell cannot be used with this procedure.
SulfaVer® 4 contains barium chloride. The final solution will contain barium chloride (D005) at a concentration regulated as a hazardous waste by the Federal RCRA. Refer to a current MSDS for safe handling and disposal instructions.
Use a blank AccuVac® Ampule in place of the sample cell in Step 5 .if necessary.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
SulfaVer® 4 Reagent Powder Pillows	1
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
SulfaVer® 4 Reagent AccuVac® Ampuls	1
Beaker, 50-mL	1
Sample Cells (see *Instrument-specific information*)	1
Stopper	1

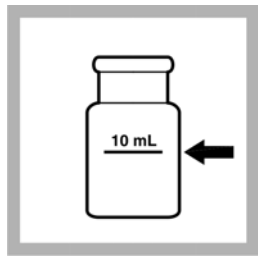
See *Consumables and replacement items* for reorder information.

SulfaVer 4 powder pillow procedure



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*). Refer to the user manual for orientation.



2. **Prepared sample:** Fill a sample cell with 10 mL of sample.

3. Add the contents of one SulfaVer 4 Reagent Powder Pillow to the sample cell. Swirl vigorously to dissolve the powder.

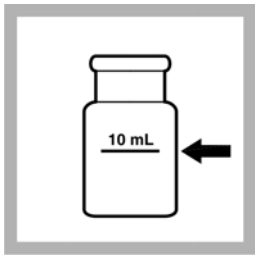
White turbidity will form if sulfate is present.

4. Start the instrument timer.

A five-minute reaction time will begin. Do not disturb the cell during this time.

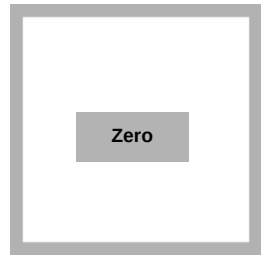
Note: *Accuracy is not affected by undissolved powder.*

SulfaVer 4 powder pillow procedure (continued)



5. Blank preparation: Fill a second sample cell with 10 mL of sample.

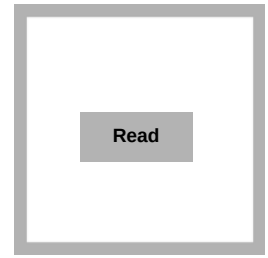
6. When the timer expires, wipe the blank and insert it in the cell holder (fill lines face right.)



7. ZERO the instrument.

The display will show:

0 mg/L SO_4^{2-}

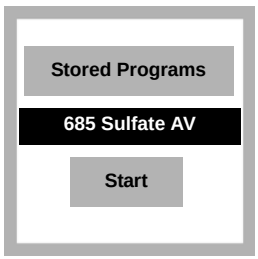


8. Within five minutes after the timer expires, wipe the cell and insert the prepared sample in the cell holder.

READ the results in mg/L SO_4^{2-}.

Clean sample cells with soap and a brush.

SulfaVer 4 AccuVac® Ampuls procedure



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*). Refer to the user manual for orientation.

2. Prepared sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill a SulfaVer 4 Reagent AccuVac® Ampul with sample from the beaker. Keep the tip immersed while the Ampul fills completely.

3. Cap or stopper the Ampul and quickly invert several times to mix.

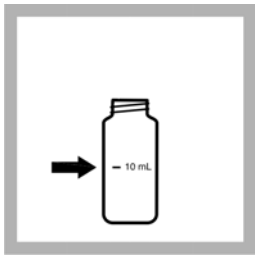
White turbidity will form if sulfate is present.

4. Start the instrument timer.

A five-minute reaction time will begin. Do not disturb the cell during this time.

Note: *Accuracy is not affected by undissolved powder.*

SulfaVer 4 AccuVac® Ampuls procedure (continued)



5. Blank Preparation: Fill a clean sample cell with 10 mL of sample.

6. When the timer expires, wipe the blank and insert it in the cell holder.

ZERO the instrument.

The display will show:

0 mg/L SO_4^{2-}

7. Within five minutes after the timer expires, wipe the Ampul and insert it in the cell holder.

READ the results in mg/L SO_4^{2-}.

Interferences

Table 344 Interfering substances

Interfering substance	Interference level
Calcium	Greater than 20,000 mg/L as $CaCO_3$
Chloride	Greater than 40,000 mg/L as Cl^-
Magnesium	Greater than 10,000 mg/L as $CaCO_3$
Silica	Greater than 500 mg/L as SiO_2

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Samples may be stored up to 7 days by cooling to 4 °C (39 °F) or lower. Warm to room temperature before analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Sulfate Ampule Standard Solution, 2500 mg/L sulfate
- Ampule breaker
- TenSette Pipet and pipet tips
- Mixing cylinder, 25 mL or 50 mL
- Beaker, 50 mL

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS**>**MORE**>**STANDARD ADDITIONS**.

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.

4. Open the standard solution ampule.

5. Fill three mixing cylinders with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to each mixing cylinder and mix thoroughly. Transfer 10 mL of each sample spike to a clean sample cell.

   Note: *For AccuVac® Ampuls, fill three mixing cylinders with 50 mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.*

6. Follow the *SulfaVer 4 powder pillow procedure* for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.

   Note: *For AccuVac Ampuls, follow the SulfaVer 4 AccuVac® Ampuls procedure for each of the spiked samples, starting with the 0.2 mL sample spike. Measure each of the spiked samples in the instrument.*

7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Required for accuracy check:

- Sulfate standard solution, 1000 mg/L
- 100 mL Class A volumetric flask
- Tensette pipet, 1–10 mL and pipet tips

1. Prepare a 70 mg/L sulfate standard solution as follows: use a pipet to add 7.0 mL of sulfate standard solution, 1000 mg/L as SO_4^{2-}, to a 100-mL volumetric flask. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.

2. Follow the *SulfaVer 4 powder pillow procedure* or *SulfaVer 4 AccuVac® Ampuls procedure* and use the 70-mg/L SO_4^{2-} standard solution in place of the sample.

3. To adjust the calibration curve using the reading obtained with the standard solution, set standard adjust to on (**OPTIONS**>(**MORE**)>**STANDARD ADJUST)** and accept the concentration.

Calibration

A calibration is recommended for the SulfaVer 4 method for the best accuracy. Complete the following steps to enter a new calibration curve in the instrument. Perform this procedure for each new lot of reagent.

Required items:

- Sulfate standard solution, 1000 mg/L
- Seven 100 mL Class A volumetric flasks
- 1–10 mL TenSette pipet and tips

1. Prepare seven calibration standards (10, 20, 30, 40, 50, 60 and 70 mg/L SO_4^{2-}) as follows. Use the Tensette pipet to add 1, 2, 3, 4, 5, 6 and 7 mL of the 1000-mg/L sulfate standard solution to seven different 100-mL Class A volumetric flasks.

2. Dilute each flask to the mark with deionized water. Mix thoroughly.

3. Use each standard solution in place of the sample and follow the *SulfaVer 4 powder pillow procedure* or *SulfaVer 4 AccuVac® Ampuls procedure*.

4. Refer to the user manual (user programs section) to enter the calibration in the instrument as a user program.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
680	40 mg/L SO_4^{2-}	30–50 mg/L SO_4^{2-}	0.4 mg/L SO_4^{2-}
685	40 mg/L SO_4^{2-}	32–48 mg/L SO_4^{2-}	0.7 mg/L SO_4^{2-}

Summary of method

Sulfate ions in the sample react with barium in the SulfaVer 4 and form a precipitate of barium sulfate. The amount of turbidity formed is proportional to the sulfate concentration. Test results are measured at 450 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
SulfaVer® 4 Reagent Powder Pillows	1	100/pkg	2106769
OR			
SulfaVer® 4 Sulfate Reagent AccuVac® Ampuls	1	25/pkg	2509025

Required apparatus

Description	Quantity	Unit	Catalog number
AccuVac snapper	1	each	2405200
Beaker, 50-mL	1	each	50041H
Sample cell, 10 mL, round, 25 x 54 mm	1	each	2122800
Sample cell, 10 mL round, 25 x 60 mm	1	each	2427606
Sample cell, 10 mL, square, matched pair	2	2/pkg	24954025
Stopper	1	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Sulfate Standard Solution, 1000-mg/L	500 mL	2175749
Sulfate Standard Solution, 2500-mg/L, 10-mL Ampules	16/pkg	1425210
Mixed Parameter Standard for sulfate, fluoride, nitrate and phosphate	500 mL	2833049

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25-mL	each	189640
Cylinder, mixing, 50-mL	each	189641
Blank AccuVac Ampules	25/pkg	2677825
Ampule Breaker, for 10 mL Ampules	each	2196800
Tensette Pipet 0.1–1.0 mL	each	1970001
Tips for Tensette Pipet 1–10	50/pkg	2185696
Tensette Pipet 1–10	each	1970010
Tips for tensette Pipet 1–10	50/pkg	2199796
Flask, volumetric, Class A, 100-mL	each	1457442

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Edition 7

Sulfide

DOC316.53.01136

USEPA[1] Methylene Blue Method[2] — Method 8131

(5 to 800 µg/L)

Scope and Application: For testing total sulfides, H_2S, HS^-, and certain metal sulfides in groundwater, wastewater, brines and seawater.

[1] USEPA approved for reporting wastewater analysis. Procedure is equivalent to Standard Method 4500-S^{2-} D.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 345 Instrument-specific information

Instrument	Sample volume	Sample cell	Cell orientation
DR 6000	10 mL	2495402	Fill line faces right
DR 5000	10 mL	2495402	Fill line faces user
DR 3900	10 mL	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	10 mL	2495402	Fill line faces right

Before starting the test:

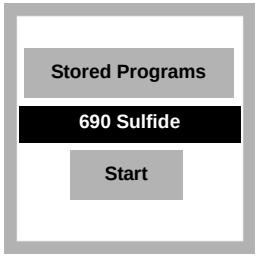
Analyze samples immediately. Do not preserve for later analysis.
Avoid excessive agitation of samples to minimize sulfide loss.
Some sulfide loss may occur if dilution is necessary.
Sulfide 2 reagent contains potassium dichromate. The final solution will contain hexavalent chromium (D007) at a concentration that is regulated as a hazardous waste by Federal RCRA. Refer to the current MSDS for safe handling and disposal instructions.

Collect the following items:

Description	Quantity
Sulfide 1 Reagent	1–2 mL
Sulfide 2 Reagent	1–2 mL
Water, deionized	10–25 mL
Pipet, serological, 10-mL	1
Pipet Filler, safety bulb	1
Sample Cells (see *Instrument-specific information*)	2
Stoppers	2

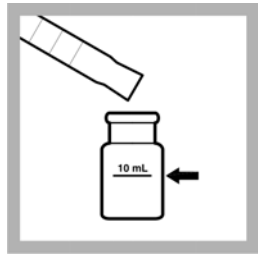
See *Consumables and replacement items* for reorder information.

Methylene Blue Method

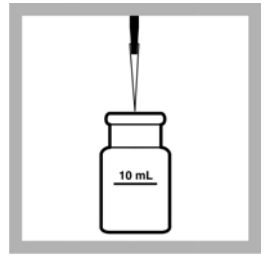


1. Select the test.

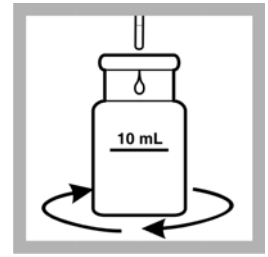
Insert an adapter if required (see *Instrument-specific information*). Refer to the user manual for orientation.



2. Blank Preparation: Measure 10 mL of deionized water in a sample cell.



3. Prepared Sample: Use a pipet to add 10 mL of sample to a second sample cell. Do not mix the sample more than necessary to prevent sulfide loss.



4. Use the dropper to add 0.5 mL Sulfide 1 Reagent to each cell.

Swirl to mix.

5. Use the dropper to add 0.5 mL Sulfide 2 Reagent to each cell.

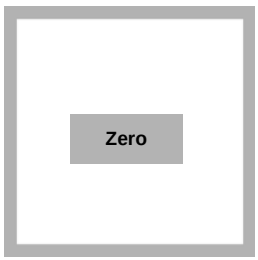
6. Cap or stopper the cell and immediately invert to mix.

The solution will turn pink initially and then turn blue if sulfide is present.

7. Start the instrument timer.

A five-minute reaction time will begin.

8. When the timer expires, wipe the blank and insert it in the cell holder.

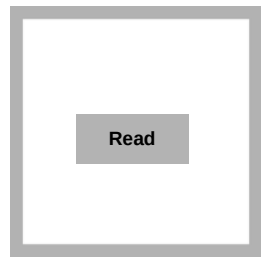


9. **ZERO** the instrument.

The display will show:

0.00 µg/L S^{2-}

10. Wipe the prepared sample and insert it in the cell holder.



11. **READ** the results in µg/L S^{2-}.

Soluble sulfides

Complete the following steps to measure soluble sulfides.

1. Centrifuge a sample in completely filled, capped tubes.
2. Use the supernatant in place of the sample and follow the *Methylene Blue Method* procedure.

To estimate insoluble sulfides, subtract the soluble sulfide concentration from the total sulfide concentration.

Interferences

Table 346 Interfering substances

Interfering substance	Interference level
Strong reducing substances such as sulfite, thiosulfate and hydrosulfite.	Interfere by reducing the blue color or preventing its development.
Sulfide, high levels	High concentrations of sulfide may inhibit full color development and require sample dilution. Some sulfide loss may occur when the sample is diluted.
Turbidity	For turbid samples, prepare a sulfide-free blank as follows. Use this blank in place of the deionized water blank in the *Methylene Blue Method* test procedure. 1. Measure 25 mL of sample into a 50-mL Erlenmeyer flask. 2. Add bromine water by drops with constant swirling until a permanent yellow color just appears. 3. Add phenol solution by drops until the yellow color just disappears. Use this solution to replace the deionized water in step 2 of the procedure. This pretreatment procedure removes sulfide from the sample, but the turbidity and any color will remain. The interference from turbidity or color will be corrected when the instrument is set to zero with this solution (step 9).

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Prevent excessive shaking or prolonged exposure to air. Analyze samples immediately.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
690	DR 5000	520 µg/L S^{2-}	504–536 µg/L S^{2-}	5µg/L S^{2-}

Summary of method

Hydrogen sulfide and acid-soluble metal sulfides react with N,N-dimethyl-p-phenylenediamine sulfate to form methylene blue. The intensity of the blue color is proportional to the sulfide concentration. High sulfide levels in oil field waters may be determined after proper dilution. Test results are measured at 665 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Sulfide Reagent Set, includes:	—	—	2244500
Sulfide 1 Reagent	1 mL	100 mL MDB	181632
Sulfide 2 Reagent	1 mL	100 mL MDB	181732
Water, deionized	10 mL	4 liters	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Pipet, serological, 10-mL	1	each	53238
Pipet Filler, safety bulb	1	each	1465100
Stopper, for 18-mm Tube	2	6/pkg	173106

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water, 30 g/L	29 mL	221120
Phenol Solution, 30 g/L	29 mL	211220
Stopper, for 18-mm Tube	25/pkg	173125
Flask, Erlenmeyer, 50 mL	each	50541

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Edition 7

Sulfite

DOC316.53.01162

Iodate-Iodide Buret Titration Method[1]

Method 8071

0 to 500 mg/L as SO_3^{2-} (or 0 to > 500 mg/L)

Buret Titration

Scope and Application: For boiler water.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

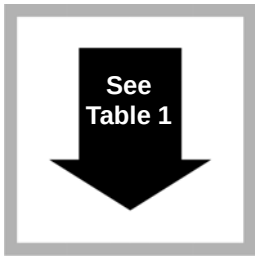
Sulfite is easily destroyed by atmospheric oxygen. Violent shaking or swirling will cause low results.
Analyze samples immediately. Cool hot samples to 50 °C (122 °F) or lower before analysis.

Collect the following items:

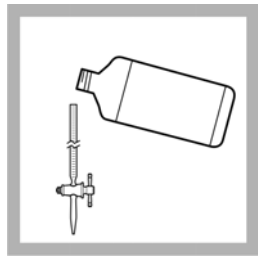
Description	Quantity
Dissolved Oxygen Reagent Powder Pillow	1 pillow
Potassium Iodide-Iodate Standard Solution, 0.0125 N	1 bottle
Starch Indicator Solution	1 bottle
Buret, Class A, 10-mL, with support stand	1
Graduated cylinder (see *Range-specific information*)	1
Erlenmeyer flask, 250-mL	1

See *Consumables and replacement items* for reorder information.

Buret titration



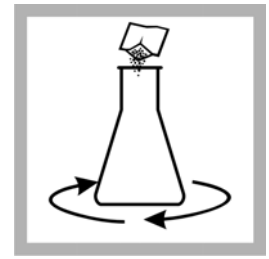
1. Select a sample volume from the *Range-specific information*.



2. Fill the buret to the zero mark with 0.0125 N Potassium Iodide-Iodate Standard Solution.

3. Use a graduated cylinder or pipet to measure the sample volume. Add the sample to the Erlenmeyer flask.

If the sample volume is less than 50 mL, gently dilute to approximately 50 mL with deionized water. Do not agitate.



4. Add the contents of one Dissolved Oxygen 3 Powder Pillow and mix gently.

Buret titration (continued)

5. Add one full dropper of Starch Indicator Solution. Swirl gently to mix.

6. Titrate the sample while gently swirling the flask until the color changes to a permanent blue color (end point).

Write down the volume of titrant that was used.

7. Use the multiplier in the *Range-specific information* to calculate the concentration:

mL titrant used x multiplier = mg/L sulfite (SO_3^{2-})

Example: 50 mL of sample was titrated and 5 mL of titrant was used to reach the end point. The concentration is 5 x 10 = 50 mg/L as SO_3^{2-}

Table 347 Range-specific information

Range (mg/L as SO_3^{2-})	Sample volume (mL)	Multiplier
0–100	50	10
40–200	25	20
100–500	10	50
Over 500	5	100

Interferences

Interfering substances lists substances that can interfere with this test.

Table 348 Interfering substances

Interfering substance	Interference level
Nitrite	Nitrite will react with sulfite to cause a negative error in the titration.
Metals	Some metals, especially copper, catalyze the oxidation of sulfite to sulfate. Immediate fixing of sample with the contents of one Sulfamic Acid Powder Pillow per liter of sample will help minimize the interference.
Organic compounds	Oxidizable organic compounds could cause high results.
Oxidizable compounds	Will cause high results.
Sulfide	Will cause high results.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles. Fill completely and cap tightly.
- Avoid excessive agitation or prolonged exposure to air. Complete the test procedure as soon as possible after collection for best accuracy. Samples cannot be preserved or stored.
- Cool hot samples to 50 °C (122 °F) or lower.

Accuracy check

The standard additions method can be used to find if the sample has an interference. The standard solution method can be used to confirm analytical technique and reagent performance.

Standard additions method (sample spike)
Required for accuracy check:

- Sulfite Voluette Ampule Standard, 5,000-mg/L SO_3^{2-}
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL and Pipet Tips
- Pipet filler

1. Open the standard solution ampule.
2. Use the TenSette Pipet to add 0.2 mL of the standard to the titrated sample. Swirl to mix.
3. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
4. Use the TenSette Pipet to add 0.3 mL of standard to the titrated sample. Swirl to mix.
5. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
6. Use the TenSette Pipet to add 0.4 mL of standard to the titrated sample. Swirl to mix.
7. Titrate the spiked sample to the end point. Write down the amount of titrant that was used to reach the end point.
8. Each 0.1 mL of standard that was added will use 1.0 mL of titrant to reach the endpoint. If more or less titrant was used, there can be an interference (see *Interferences*) or the concentration of the titrant has changed (see *Standard solution method*).

Standard solution method
Complete the following test to make sure the concentration is accurate.

Required for accuracy check:

- 0.025 N Sodium Thiosulfate Standard Solution
- 250-mL volumetric flask, Class A
- 10-mL volumetric pipet, Class A

1. Use a pipet to add 10.0-mL of 0.025 N Sodium Thiosulfate Standard Solution to a 250-mL volumetric flask. Dilute to the mark with deionized water. The diluted standard is equivalent to 40 mg/L sulfite.
2. Use a graduated cylinder to measure 50 mL of the diluted standard. Add the standard to the Erlenmeyer flask.
3. Titrate the standard to the end point with the titrant solution. The result should be close to 40 mg/L sulfite.

Summary of method

The water sample is acidified, treated with a starch indicator and titrated with a potassium iodide-iodate standard solution. The acid releases free iodine, which is reduced to colorless iodide by the sulfite in the sample. When all of the sulfite is gone, excess iodine will react with the starch to form a blue color.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Dissolved Oxygen 3 Reagent Powder Pillows	1 pillow	100/pkg	98799
Potassium Iodide-Iodate Standard Solution, 0.0125 N	varies	1 L	1400153
Starch Indicator Solution	1 mL	100 mL MDB	34932
Reagent set, Sulfite Buret Titration	—	100 tests	2459800

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, Teflon Stopcock plug, Certified 10-mL	1	each	2636538
Buret Clamp, double	1	each	32800
Clippers, for opening pillows	1	each	96800
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 5-mL	1	each	50837
Cylinder, graduated, 10-mL	1	each	50838
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Support Stand	1	each	56300
Pipet, volumetric, Class A, 5 mL	1	each	1451537
Pipet, volumetric, Class A, 10 mL	1	each	1451538
Pipet filler, Safety Bulb	1	each	1465100

Recommended standards

Description	Unit	Catalog number
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	2409353
Sulfite Standard Solution, Voluette® Ampule, 5,000-mg/L SO_3 10-mL	16/pkg	1426710

Optional reagents and apparatus

Description	Unit	Catalog number
TenSette Pipet, 0.1 to 1.0 mL	each	1970001
Pipet tips	50/pkg	2185696
Ampule breaker	each	2196800

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Edition 7

Sulfite

DOC316.53.001181

Iodate-Iodide Method **Method 8216**

4 to greater than 400 mg/L as SO_3^{2-} **Digital Titrator**

Scope and Application: For boiler water

Test preparation

Before starting the test:

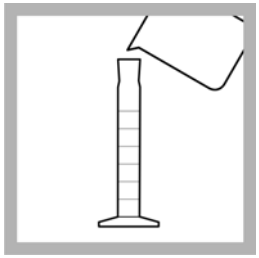
Analyze samples immediately. Cool hot samples to 50 °C (122 °F) or lower before analysis.
Sulfite is easily destroyed by atmospheric oxygen. Violent shaking or swirling will cause low results. Avoid unnecessary agitation throughout the procedure.
Use the TitraStir apparatus for best results.
The Dissolved Oxygen 3 Reagent Powder Pillow can be replaced with 0.5 mL of 19.2 N Sulfuric Acid Standard Solution.
mg/L Sulfite (SO_3^{2-}) x 1.01 = Bisulfite, Hydrogen Sulfite (HSO_3^-)
mg/L Sulfite (SO_3^{2-}) x 1.30 = Sodium Bisulfite, Sodium Hydrogen Sulfite ($NaHSO_3$)
mg/L Sulfite (SO_3^{2-}) x 2.37 = Sodium Metabisulfite, Sodium Pyrosulfite ($Na_2S_2O_5$)
mg/L Sulfite (SO_3^{2-}) x 1.58 = Sodium Sulfite (Na_2SO_3)

Collect the following items:

Description	Quantity
Sulfite Reagent Set	1
Clippers	1
Deionized Water	varies
Digital Titrator	1
Erlenmeyer Flask, 125-mL	1
Graduated Cylinder, 10-, 25- or 50-mL, based on sample concentration	varies

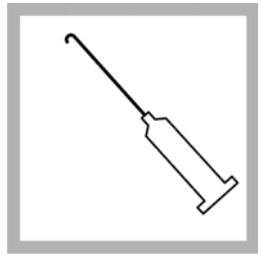
See *Consumables and replacement items* for reorder information.

Iodate-Iodide Method



8. Select a sample volume from the *Volume multipliers* table that corresponds to the expected sulfite (SO_3^{2-}) concentration.

Use a graduated cylinder to measure the sample volume from the *Volume multipliers* table.



9. Insert a clean delivery tube into the Iodate-Iodide Titration Cartridge (KIO_3–KI).

Attach the cartridge to the titrator body.

10. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

11. Transfer the sample volume into a clean, 125-mL Erlenmeyer flask. Dilute to the 50-mL mark with deionized water.

12. Add the contents of one Dissolved Oxygen 3 Reagent Powder Pillow and swirl gently to mix.

13. Add one dropperful of Starch Indicator Solution and swirl to mix.

14. Place the delivery tube tip into the solution and swirl the flask while titrating with the iodate-iodide to a permanent blue end point. Record the number of digits required.

15. Calculate:
Digits Required x Digit Multiplier = mg/L Sulfite (SO_3^{2-})

Table 349 Volume multipliers

Range (mg/L as SO_3^{2-})	Volume (mL)	Cartridge Strength	Digit Multiplier
Up to 160	50	0.3998 N	0.4
100–400	20	0.3998 N	1.0
Over 400	5	0.3998 N	4.0
200–800	10	0.3998 N	2.0

Accuracy check

Standard additions method (Sample spike)
This accuracy check should be performed when interferences are suspected or to verify analytical technique.

1. Snap the top off a Sulfite Voluette Ampule Standard, 5,000-mg/L SO_3^{2-}.
2. Use a TenSette Pipet to add 0.1 mL of standard to the sample titrated. Resume titration back to the same end point. Record the number of digits required.
3. Repeat, using additions of 0.2 and 0.3 mL, titrating to the end point after each.
4. Each 0.1-mL addition of standard should require 25 additional digits of titrant. If these uniform increases do not occur, determine the cause.

A standard solution equivalent to 40-mg/L sulfite can be prepared by diluting 10.0 mL of 0.025 N Sodium Thiosulfate Titrant to 250 mL in a volumetric flask. Titrate a 50-mL sample, using the above procedure.

Interferences

- Sulfide, organic matter and other oxidizable substances will cause positive error in the titration.
- Nitrite will react with sulfite to cause low results.
- Some metals, especially copper, catalyze the oxidation of sulfite to sulfate.
- Addition of one Dissolved Oxygen 3 Powder Pillow per liter of sample immediately upon sampling will help eliminate the effects of nitrite and copper.

Summary of method

Sulfite ion is titrated with potassium iodate-iodide standard solution under acidic conditions to a blue starch end point. The volume of titrant used is proportional to the sulfite concentration.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Sulfite Reagent Set (about 100 tests), includes:		2272300
Dissolved Oxygen 3 Reagent Powder Pillows	100/pkg	98799
Iodate-Iodide Titration Cartridge, 0.3998 N	each	1496101
Starch indicator Solution	100 mL MDB	34932
Water, deionized	4 L	27256

Required apparatus

Description	Unit	Catalog number
Clippers for opening pillows	each	96800
Digital Titrator	each	1690001
Cylinder, graduated, 10-mL	each	50838
Cylinder, graduated, 25-mL	each	50840
Cylinder, graduated, 50-mL	each	50841
Flask, Erlenmeyer, 125-mL	each	50543
Delivery tubes w/ 180° hook	each	1720500
Delivery tubes w/ 90° hook	each	4157800
Thermometer -10–225 °C, 405 mm	each	2635700
Volumetric Pipet, 10 mL	each	1451538
Volumetric flask, 250 mL	each	1457446
Safety bulb	each	1465100
Tensette Pipet	each	1970001
Pipet tips	50/pkg	2185696
Pipet tips	1000/pkg	2185628

Required standards

Description	Unit	Catalog number
Sodium Thiosulfate Standard Solution, 0.025 N	1000 mL	2409353
Sulfite Standard Solution, Voluette® Ampule, 5,000-mg/L SO_3 10-mL	16/pkg	2267410
Sulfuric Acid Standard Solution, 19.2 N	100 mL MDB	203832
Ampule Breaker	each	2196800
Sulfite Standard Solution, 15 mg/L	500 mL	2408449

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Edition 7

Sulfite

DOC316.53.01137

Colorimetric Method[1]

(0.10 to 5.00 mg/L)

Scope and Application: For boiler water and foodstuffs.

[1] Reagent sets for this method are only available in Europe.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test

Table 350 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

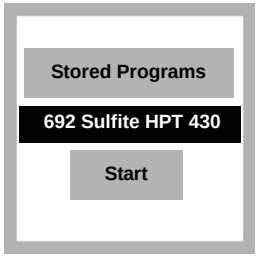
Samples must be analyzed immediately.
The temperature of samples and reagents must be between 15–25 °C (59–77 °F).
Adjust the pH of the sample to a value between 3–10.

Collect the following items:

Description	Quantity
Sulfite Reagent A	5 drops
Sulfite Reagent B	2 drops
Water, deionized	varies
Pipet, 10-mL serological	1
Pipet Filler, safety bulb	1
Sample Cells (see the *Instrument-specific information* table)	2

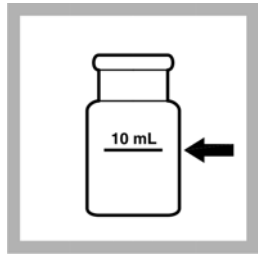
See *Consumables and replacement items* for reorder information.

Colorimetric Method

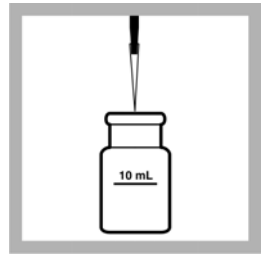


1. Select the test.

Insert an adapter if required (see the *Instrument-specific information* table). Refer to the user manual for orientation.



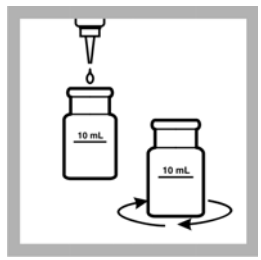
2. Blank Preparation: Fill a clean sample cell with 10 mL of sample.



3. Prepared Sample: Pipet 10 mL of sample into a second clean sample cell.

4. Add 5 drops of Sulfite Reagent A (HPT 430 A) to the prepared sample.

5. Swirl to mix.

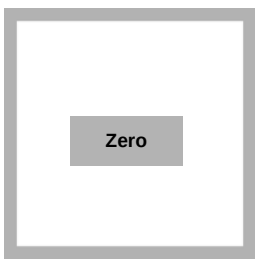


6. Add 2 drops of Sulfite Reagent B (HPT 430 B) to the prepared sample. Swirl to mix.

7. Start the instrument timer.

A 3-minute reaction time will begin. Do not disturb the cell during this time.

8. Wipe the blank and insert it in the cell holder.

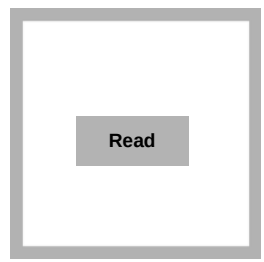


9. **ZERO** the instrument.

The display will show:

0.00 mg/L SO_3^{2-}

10. When the timer expires, wipe the prepared sample and insert it in the cell holder.



11. **READ** the results in mg/L SO_3^{2-}.

Interferences

Table 351 Interfering substances

Interfering substance	Interference level
Sulfide	Greater than 5 mg/L

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Analyze immediately.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
692	DR 5000	3.00 mg/L SO_3^2	2.51–3.49 mg/L SO_3^{2-}	0.04 mg/L SO_3^{2-}

Summary of method

The reagents react with sulfite to form a yellow complex. The color is measured at 435 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Sulfite Colorimetric Reagent Set, includes:	—	100/pkg	HPT430
Sulfite Reagent A[1]	5 drops	28 mL	—
Sulfite Reagent B[1]	2 drops	8.7 mL	—

[1] Not available separately. Reagent sets for this method are only available in Europe.

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Pipet, 10-mL serological	1	each	53238
Pipet Filler, safety bulb	1	each	1465100

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Edition 7

Surfactants, Anionic (Detergents) DOC316.53.01138

Crystal Violet Method[1] **Method 8028**

(0.002 to 0.275 mg/L as LAS)

Scope and Application: For water, wastewater and seawater.

[1] Adapted from *Analytical Chemistry, 38, 791 (1966)*.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays information that may vary from instrument to instrument. Select the spectrophotometer from the instrument column on the left. Read across to find the corresponding sample cells and adapters required to perform this test on the spectrophotometer.

Table 352 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2612602	Fill line faces right
DR 5000	2612602	Fill line faces user
DR 3900	2612602	Fill line faces user
DR 3800, DR 2800, DR 2700	2612602	Fill line faces right

Before starting the test:

Use benzene only in a well-ventilated area.

Benzene (D018) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Collect water that is saturated with benzene and benzene solutions for disposal with laboratory solvent wastes. Refer to the current MSDS for safe handling and disposal instructions.

To prevent water droplets from forming in the sample cells, use only dry sample cells and discard the first few mL of benzene. Additionally, it can help to transfer the liquid from the funnel to a sample cell, let it sit for a few seconds and decant to a second cell for reading.

Excessive shaking may cause an emulsion to form, which makes the phases separate more slowly. If this occurs, remove most of the water layer, then gently mix the contents of the funnel with a clean Teflon®-coated rod or other inert tool.

Spilled reagent will affect test accuracy and is hazardous to the skin and other materials.

Acetone may be used to clean benzene from glassware.

For best results, measure a reagent blank value for each new lot of reagent (follow the procedure using deionized water in place of the sample). Subtract the reagent blank value from the final results or enter the value as a reagent blank adjust for automatic subtraction.

In bright light conditions (e.g. direct sunlight) be sure to cover the cell compartment during measurements.

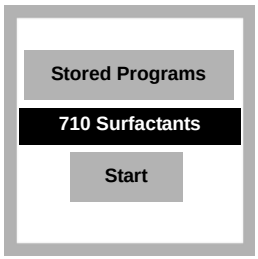
The Pour-Thru Cell cannot be used with this procedure.

Collect the following items:

Description	Quantity
Benzene, ACS	55 mL
Buffer Solution, sulfate-type	10 mL
Detergent Reagent Powder Pillows	1 pillow
Clippers, for opening powder pillows	1
Cylinder, graduated, 25-mL	1
Cylinder, graduated, 50-mL	1
Cylinder, graduated, 500-mL	1
Funnel, separatory, 500-mL	1
Sample Cells (see the *Instrument-specific information* table)	2
Support Ring, 4-inch	1
Support, Ring Stand, 5 x 8 inch base	1

See *Consumables and replacement items* for reorder information.

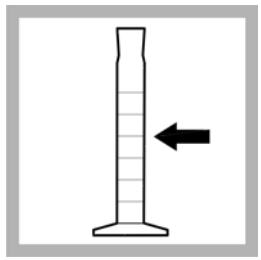
Crystal Violet Method



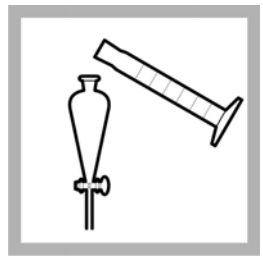
1. Select the test.

Insert an adapter if required (*Instrument-specific information* table).

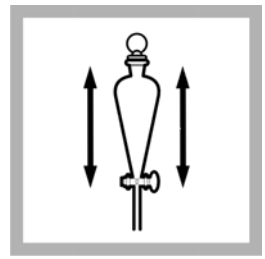
Refer to the user manual for orientation.



2. Fill a clean 500-mL graduated cylinder to the 300 mL mark with sample.



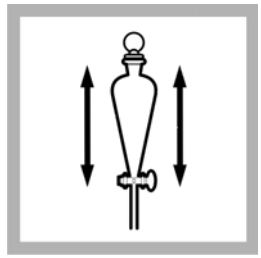
3. Pour the sample into a clean 500-mL separatory funnel.



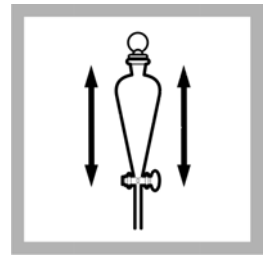
4. Add 10 mL of Sulfate Buffer Solution. Stopper the funnel. Shake the funnel for five seconds.

Crystal Violet Method (continued)

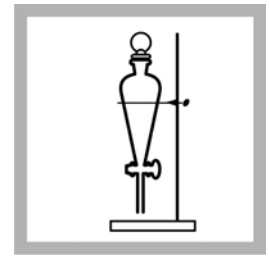
5. Add the contents of one Detergents Reagent Powder Pillow to the funnel.



6. Stopper the funnel and shake until the powder dissolves completely. The powder will dissolve slowly.



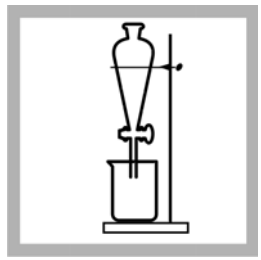
7. Add 30 mL of benzene to the funnel. Stopper the funnel and shake gently for one minute.



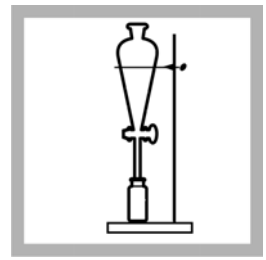
8. Place the separatory funnel in a support stand.

9. Start the instrument timer.

A 30-minute reaction period will begin.

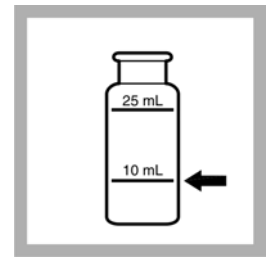


10. After the timer expires, remove the stopper and drain the bottom water layer. Discard this layer.



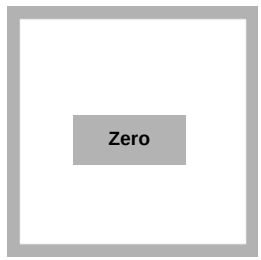
11. Prepared Sample: Drain the top benzene layer into a clean 25-mL sample cell. Stopper the cell.

Do not filter the benzene layer before color measurement. Filtration removes the blue color.



12. Blank Preparation: Fill another sample cell to the 10-mL mark with pure benzene. Stopper the cell.

13. Wipe and insert the blank cell into the cell holder.

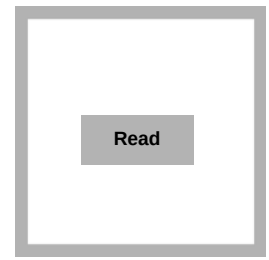


14. ZERO the instrument.

The display will show:

0.000 mg/L LAS

15. Wipe and insert the sample cell into the cell holder.



16. READ the results in mg/L LAS.

Interferences

Table 353 Interfering substances

Interfering substance	Interference level
Chloride	High amounts of chloride, such as those levels found in brines and seawater, will cause low results.
Perchlorate ions	Interferes at all levels.
Periodate ions	Interferes at all levels.

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Analyze samples as soon as possible. Samples may be stored at least 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before testing.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Detergent Voluette® Ampule Standard, 60-mg/L LAS
- Ampule breaker
- TenSette Pipet, 0.1–1.0 mL and Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Prepare three sample spikes as follows. Measure 300 mL of sample into each of the three beakers. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Follow the test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Required for accuracy check:

- Detergent Voluette® Ampule Standard, 60-mg/L LAS
- 1-L Class A volumetric flask
- 3.0 mL Class A volumetric pipet and pipet bulb

1. Prepare a 0.180 mg/L LAS standard solution as follows: Pipet 3.0 mL of Detergent Standard, 60-mg/L as LAS, into a 1000-mL (1 liter) volumetric flask. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Follow the *Crystal Violet Method* test procedure.
3. To adjust the calibration curve with the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust option and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
710	0.180 mg/L LAS	0.172–0.188 mg/L LAS	0.002 mg/L LAS

Summary of method

Detergents, ABS (alkyl benzene sulfonate) or LAS (linear alkylate sulfonate) are determined by association with crystal violet dye and extraction of the ion-pair complex into benzene. Test results are measured at 605 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Detergents Reagent Set, includes:	—	—	2446800
Benzene, ACS	40 mL	4 liters	1444017
Buffer Solution, sulfate-type	10 mL	500 mL	45249
Detergent Reagent Powder Pillows	1 pillow	25/pkg	100868

Required apparatus

Description	Quantity	Unit	Catalog number
Clippers, for opening powder pillows	1	each	96800
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 500-mL	1	each	50849
Funnel, separatory, 500-mL	1	each	52049
Support Ring, 4-inch	1	each	58001
Support, Ring Stand, 5 x 8 inch base	1	each	56300

Recommended standards

Description	Unit	Catalog number
Detergent Standard Solution, 10-mL Voluette® Ampule, 60-mg/L LAS	16/pkg	1427110

Optional reagents and apparatus

Description	Unit	Catalog number
Acetone, ACS	500 mL	1442949
Beaker, 600 mL	each	50052
Flask, volumetric, Class A, 1000 mL	each	1457453
Pipet filler bulb	each	1465100
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet tips for TenSette Pipet 19700-01	50/pkg	2185696
Pipet, volumetric, Class A, 3.00 mL	each	1451503

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Edition 7

Tannin and Lignin

DOC316.53.01140

Tyrosine Method[1]

Method 8193

(0.1 to 9.0 mg/L)

Scope and Application: For water, wastewater and boiler water.

[1] *Adapted from Kloster, M.B., Journal American Water Works Association, Vol. 66, No. 1, p. 44 (1974).*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 354 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Filter turbid samples and report results as mg/L soluble tannic acid.
For best accuracy, use a pipet to add the TanniVer® 3 solution.
The Pour-Thru Cell can be used as an alternative to the sample cells, when using a full 25 mL sample..
Results will be given in mg/L tannins (as tannic acid).

Collect the following items:

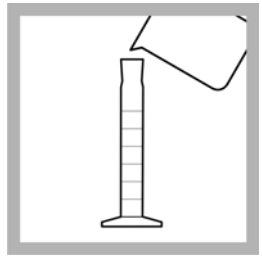
Description	Quantity
Tannin and Lignin Reagent Set:	
Sodium Carbonate Solution	10 mL
TanniVer® 3 Tannin-Lignin Reagent	1 mL
Cylinder, graduated mixing, 25-mL	2
Pipet Filler	1
Pipet, volumetric Class A, 5.0-mL	1
Pipet, volumetric Class A, 0.5-mL	1
Sample Cells (see *Instrument-specific information*)	2
Water, deionized	25 mL

See *Consumables and replacement items* for reorder information.

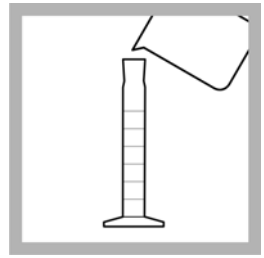
Tyrosine Method

1. Select the test.

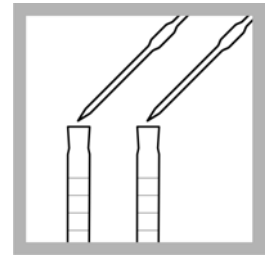
Insert an adapter if required (*Instrument-specific information*).



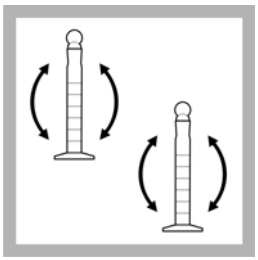
2. Blank Preparation: Fill a 25-mL graduated mixing cylinder to the 25-mL mark with deionized water.



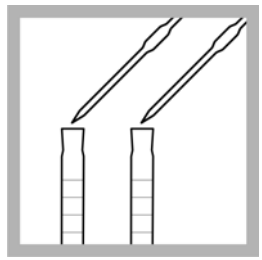
3. Prepared Sample: Fill a second 25-mL graduated mixing cylinder to the 25-mL mark with sample.



4. Pipet 0.5 mL of TanniVer® 3 Tannin-Lignin Reagent into each cylinder.

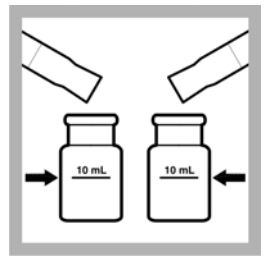


5. Insert the stopper and invert to mix.



6. Pipet 5.0 mL of Sodium Carbonate Solution into each cylinder. Insert the stopper and invert to mix.

A blue color will develop if tannins and/or lignins are present.

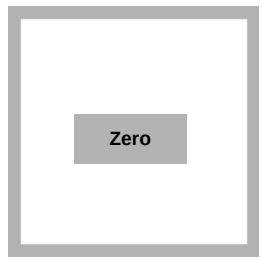


7. Pour 10 mL of each solution into two sample cells.

8. Start the instrument timer.

A 25-minute reaction period will begin.

9. When the timer expires, insert the blank into the cell holder.

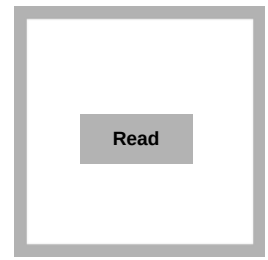


10. ZERO the instrument.

The display will show:

0.0 mg/L Tannins (as Tannic Acid).

11. Insert the sample into the cell holder.



12. READ the results in mg/L Tannins (as Tannic Acid).

Interferences

Table 355 Interfering substances

Interfering substance	Interference level
Ferrous iron	Causes a positive interference. (2 mg/L of ferrous iron produces a color equivalent to about 1 mg/L of tannic acid.) To eliminate interference of ferrous iron up to 20 mg/L, add one 0.2 g scoop of Sodium Pyrophosphate[1] to the sample before testing.
Sulfite	To eliminate sulfite interference, add 1 mL of formaldehyde[1] to the sample before testing the sample.

[1] See *Optional reagents and apparatus*.

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles.

Accuracy check

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Tannic acid, analytical grade
- 1-L Class A volumetric flask
- 500-mL Class A volumetric flask
- 15-mL Class A volumetric pipet and pipet bulb
- Deionized water

1. Prepare a tannic acid stock solution as follows: dissolve 0.200 grams of tannic acid in deionized water and dilute to 1000 mL. Prepare this solution monthly.
2. Prepare a 6.0-mg/L tannic acid standard solution by diluting 15.00 mL of the stock solution to 500 mL with deionized water. Prepare this standard daily.
3. Follow the tannin and lignin procedure as described above.
4. To adjust the calibration curve using the reading obtained with the 6.0-mg/L Standard Solution, navigate to Standard Adjust in the software:**OPTIONS**>**MORE**>**STANDARD ADJUST**.
5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
720	6.0 mg/L tannic acid	5.8–6.2 mg/L tannin	0.1 mg/L tannin

Summary of method

This test measures all hydroxylated aromatic compounds, including tannin, lignin, phenol and cresol. This method produces a blue color proportional to the amount of these compounds present

in the sample. The results are reported as total tannin and lignin and expressed as mg/L tannic acid. Test results are measured at 700 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Tannin and Lignin Reagent Set (up to 100 tests), includes:			2244600
(2) Sodium Carbonate Solution	10 mL	500 mL	67549
(1) TanniVer® 3 Tannin-Lignin Reagent	1 mL	100 mL	256032
Water, deionized	25 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, mixing, with stopper, 25-mL	2	each	2088640
Pipet filler, safety bulb	1	each	1465100
Pipet, volumetric, Class A, 5.0-mL	1	each	1451537
Pipet, volumetric, Class A, 0.5-mL	1	each	1451534

Recommended standards

Description	Unit	Catalog number
Tannic Acid, Analytical Grade	113 g	79114

Optional reagents and apparatus

Description	Unit	Catalog number
Flask, volumetric, 1000 mL	each	1457453
Flask, volumetric, 500 mL	each	1457449
Formaldehyde	100 mL	205932
Pipet, volumetric, 15.0 mL	each	1451539
Sodium Pyrophosphate	50 g	1429525
Pipet, TenSette, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Analytical balance, 80 g capacity, 0.1 mg resolution	each	2936701
Weighing papers	500/pkg	1473800
Spatula, micro	each	1225600

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Edition 7

ToxTrak™ Method [1, 2] **Method 10017**

(0 to 100% Inhibition)

Scope and Application: For drinking water, wastewater and natural waters.

[1] Liu, D., Bull. Environ. Contm. Toxicol. 26, 145-149 (1981)

[2] Environmental Technology Verification ETV Program evaluated, November, 2003

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 356 Instrument-specific information

Instrument	Light shield
DR 6000, DR 5000	—
DR 3900	LZV849
DR 3800, DR 2800, DR 2700	LZV646

Before starting the test:

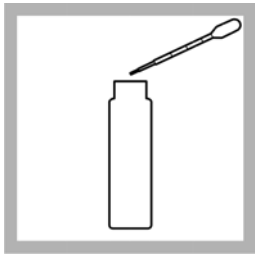
Do not leave the cells in the instrument during incubation. All samples and control cells should be allowed to react under similar conditions of temperature and light.
If testing chlorinated samples, add two drops of sodium thiosulfate to each blank and sample to remove the chlorine.
If testing drinking water, take the control sample from a reservoir of tap water known to be free of toxins, if possible.

Collect the following items:

Description	Quantity
Bacterial Count Broth Tube	1 tube
Pipet, transfer, sterile	2
Reaction tubes, with cap	1
Sodium Thiosulfate	varies
ToxTrak™ Reagent Powder Pillows	2
ToxTrak™ Accelerator Solution	4 drops
Water, deionized	varies
Clippers	1
Incubator	1
Pipet, volumetric, Class A, 5.00 mL and pipet filler	1

See *Consumables and replacement items* for reorder information.

Inoculum development using indigenous biomass



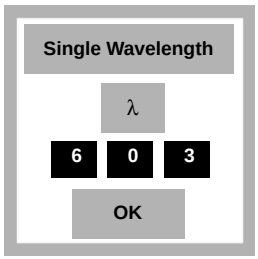
1. Using one of the dropper pipets provided, add 1.0 mL of source culture (indigenous biomass) to a Total Bacteria Count Broth Tube.

Note: *Commercial sources of freeze-dried bacteria may also be used.*

2. Incubate the tube contents at 35 °C (95 °F) until the broth is visibly turbid (approximately 12 hours).

Note: *The culture can be kept for several days in the incubator or at room temperature. Use before 72 hours for best results.*

Reaction tube colorimetric test



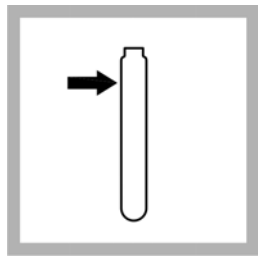
1. Press

Single Wavelength.

Press **OPTIONS** and the λ button.

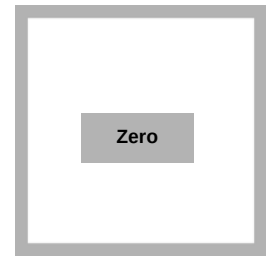
Enter **603 nm** and press **OK.**

Insert an adapter if required (*Instrument-specific information*).



2. Blank Preparation: Fill an empty reaction tube with deionized water.

3. Wipe the blank and insert it into the cell holder.



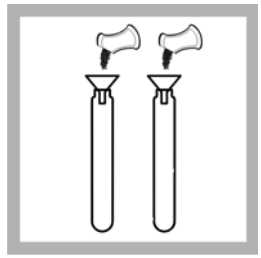
4. ZERO the instrument.

The display will show:

0.000 Abs

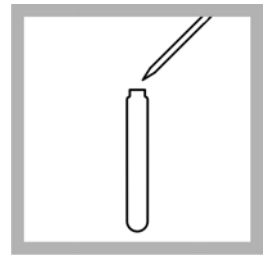
Reaction tube colorimetric test (continued)

5. Label a reaction tube “control”. Open one ToxTrak Reagent Powder Pillow and add the contents to the empty tube.



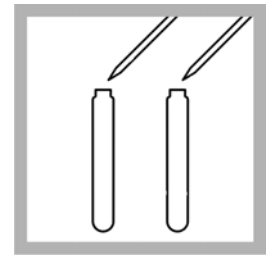
6. For each sample or dilution, label a reaction tube with the sample number.

Open one ToxTrak Reagent Powder Pillow and add the contents to each empty sample tube.



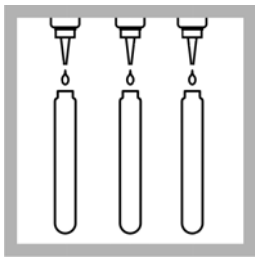
7. Add 5.0 mL of deionized water to the control tube.

Use deionized water that is free of toxicity or another water source that represents baseline toxicity.



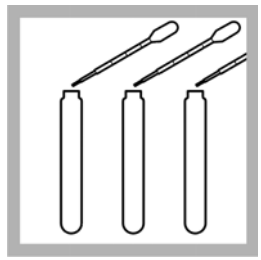
8. Add 5.0 mL of sample (or dilution) to each sample tube.

To find the approximate threshold level of toxicity for a sample, see *No observed effect concentration (NOEC)*.



9. Add two drops of Accelerator Solution to each vial. Cap and shake to mix.

Shaking fully oxygenates the samples and assures that the oxygen concentration is not a factor in determining the respiration rate.



10. Add 0.5 mL of inoculum (previously prepared) to each tube. Cap and invert to mix.

11. Insert the control in the cell holder. Record the absorbance.

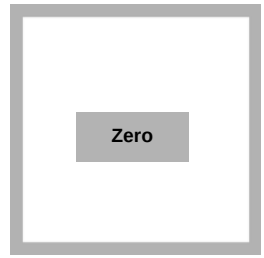
12. Repeat step 11 for all samples and dilutions. Be sure to record each absorbance.

Reaction tube colorimetric test (continued)

13. Allow the solutions in the tubes to react until the absorbance of the control has decreased by 0.60 (± 0.10) Abs. This takes 45–75 minutes. Invert occasionally.

The reaction time varies according to temperature, age of the culture, bacteria concentrations, etc.

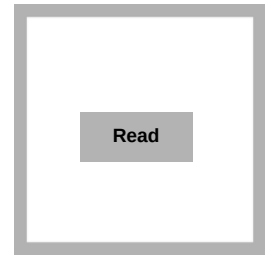
14. After the absorbance of the control has decreased by 0.60 (± 0.10) Abs. **Remove the control and insert the blank into the cell holder.**



15. **ZERO** the instrument.

The display will show:

0.000 Abs



16. Insert the control into the cell holder.

READ the results. The display will give an absorbance reading. Record this value.

17. Insert each sample or dilution into the cell holder and **READ** the results.

Record each absorbance value.

18. Calculate the % Inhibition:

$$\%\,I = \left[1 - \left(\frac{\Delta A_{sample}}{\Delta A_{control}}\right)\right] \times 100$$

where:

ΔA = Initial absorbance value – Final absorbance value

Example:

Absorbance of control: initial = 1.500 abs, final = 0.900 abs; $\Delta A_{control} = 0.600$
Absorbance of sample: initial = 1.700 abs, final = 1.300 abs; $\Delta A_{sample} = 0.400$

$$\%\,I = \left[1 - \left(\frac{0.400}{0.600}\right)\right] \times 100 = 33\%\,I$$

Interpreting results

The results as percent inhibition (% I) are a relative measurement. They do not represent a true quantitative measurement of toxic concentration. The percent inhibition does not necessarily increase in direct proportion to the concentration of toxins.

Results below 10% are not reliable, but can be used to make an estimate of toxicity when the results are consistent. If a sample shows less than 10% inhibition, repeat the test several times.

Look at the series of data points to find the likelihood of toxicity (refer to the *Interpreting results that are less than 10% inhibition* table).

Some toxins will increase respiration and give a negative percent inhibition on this and all other respiration-based toxicity tests. After repeated testing, samples that always give a percent inhibition that is more negative than –10% should be considered toxic.

Table 357 Interpreting results that are less than 10% inhibition

Data points: percent inhibition	Conclusion
7%, 9%, 5%, 8%, 5%	May be slightly toxic
7%, –4%, –5%, 5%, 1%	Most likely not toxic
–7%, –9%, –5%, –8%, –5%	May be slightly toxic

No observed effect concentration (NOEC)
To determine the minimum inhibition concentration of a toxin:

1. Dilute 1 mL of sample to 10 mL with deionized water.
2. Run the test and find the percent inhibition for the dilution.
3. Dilute 1 mL of the sample dilution from step 1 to 10 mL with deionized water.
4. Run the test and find the percent inhibition for the dilution.
5. Continue to make serial 1:10 dilutions of the sample (1:10, 1:100, 1:1000, etc.) until a level is reached that gives 0% inhibition in the final calculation.

   When 0% inhibition is found, the dilution represents the approximate threshold level of toxicity for a sample. This is the No Observed Effect Concentration (NOEC).

Lowest observable effect concentration (LOEC)
Due to the many variables involved in the test, the limit of detection is approximately 10% inhibition. This correlates to the Lowest Observable Effect Concentration (LOEC).

Disposal of test cultures

Use one of the following methods to dispose of active bacterial cultures:

- Autoclave used test containers at 121 °C (250 °F) for 15 minutes at 15 pounds of pressure. Once the containers are sterile, pour the contents down the drain with running water. The reaction tubes may be washed and re-used.
- Sterilize test containers by using a 1:10 dilution of commercial laundry bleach. Pour the test container contents and test containers into the bleach solution. Allow 10–15 minutes of contact time with the bleach solution. Then pour the liquid down the drain and wash the reaction tubes for reuse.

Summary of method

This method is based on the reduction of resazurin, a redox-active dye, by bacterial respiration. When it is reduced, resazurin changes color from blue to pink. Toxic substances can inhibit the rate of resazurin reduction. A chemical accelerant has been added to shorten the reaction time. The absorbance of the color change is measured at 603 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
ToxTrak™ Reagent Set, includes:	1	25/set	2597200
Media Set, Total Bacteria Count Tubes	1	15/pkg	2277700
Pipet, transfer, sterile	1	15/pkg	2232512
Sodium Thiosulfate Standard Solution, 0.0246 N	varies	100 mL	2409232
ToxTrak Reagent Powder Pillows	2	50/pkg	2560766
ToxTrak Accelerator Solution	4 drops	15 mL SCDB	2560836
Tubes, 16 x 100 mm (sold as 6/pkg)	1	30 tubes	2275806
Tube caps for 22758-00 (sold as 6/pkg)	1	30 caps	2241106
Water, deionized	varies	500 mL	27249

Required apparatus

Description	Unit	Catalog number
Clippers	each	93600
Dropper, 0.5 and 1.0 mL marks	20/pkg	2124720
Forceps, flat square tip	each	1453700
Incubator, Dri-Bath, 12 well, 120 VAC	each	2281400
Pipet, volumetric, Class A, 5.00 mL	each	1451537
Pipet Filler, safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips for TenSette Pipet 19700-01	50/pkg	2185696
Pipet, TenSette, 1.0 to 10.0 mL	each	1970010
Pipet Tips for TenSette Pipet 19700-10	50/pkg	2199796
Rack, test tube	each	1864100
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
BOD seed (polyseed)	50/pkg	2918700
Laboratory pen, permanent marker	each	2092000

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TPH (Total Petroleum Hydrocarbons)

DOC316.53.01142

Immunoassay[1] **Method 10050**

Scope and Application: For soil and water

[1] This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 358 Instrument-specific information

Instrument	Adapter
DR 6000	—
DR 5000	A23618
DR 3900	LZV846 (A)
DR 3800, DR 2800, DR 2700	LZV583

Before starting the test:

The TPH test can be used for both soil and water testing. When testing soil, start with the Soil Extraction Procedure. When testing water samples only, start with the Immunoassay Procedure for Soil Extracts and Water Samples. The test requires about 20 to 30 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.
Read the entire procedure before starting. Identify and make ready all the necessary reagents, cuvettes and other apparatus before beginning the analysis.
Timing is critical; follow instructions carefully.
A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the spectrophotometer.
Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
Store the reagents at 4 °C when they are not in use. Allow the reagents to reach room temperature before using them in an analysis. Actual testing may be done at temperatures ranging from 1– 38 °C.
The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.
Protective nitrile gloves are recommended for this procedure.

Collect the following items:

Description	Quantity
TPH Reagent Set	1
Caps, flip spout	1
Marker, laboratory	1
Rack, for 1-cm Micro Cuvettes	1
Wipes, disposable	1
Pipet, TenSette®, 0.1–1.0 mL	1
Pipet Tips, for TenSette Pipet 19700-01	1

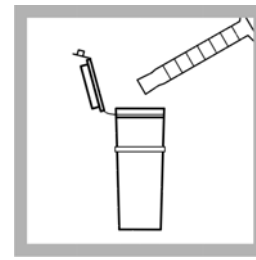
See *Consumables and replacement items* for reorder information.

Soil extraction procedure

1. Weigh out 10 g of soil in the plastic weighing boat.

2. Carefully pour the soil into an extraction vial.

3. Use the 5-gram scoop to add one scoop of sodium sulfate to the extraction vial.

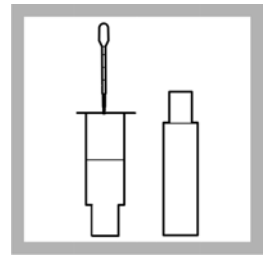


4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial.

Soil extraction procedure (continued)

5. Cap the extraction vial tightly and shake vigorously for one minute.

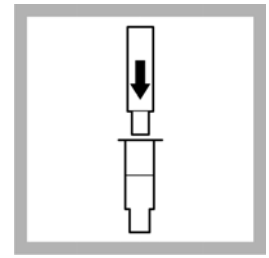
6. Allow to settle for at least one minute. Carefully open the extraction vial.



7. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid layer at the top of the extraction vial.

Transfer it into the filtration barrel (the bottom part of the filtering assembly).

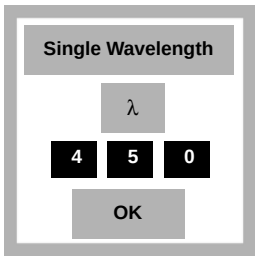
Do not use more than 1.5 mL. The bulb is marked in 0.25-mL increments.



8. Insert the filtration plunger into the filtration barrel. Place the filtration assembly on a table and press firmly on the plunger until the sample extract is forced upward into the center of the plunger.

Use the resultant filtrate as the sample in the *Immunoassay procedure for soil extracts and water samples*.

Immunoassay procedure for soil extracts and water samples



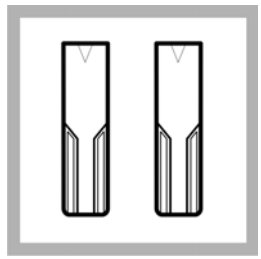
1. Press

Single Wavelength.

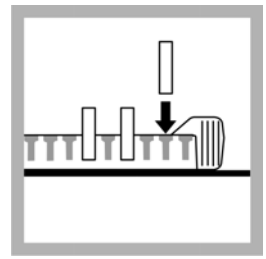
Press **OPTIONS** and the λ button.

Enter **450 nm** and press **OK.**

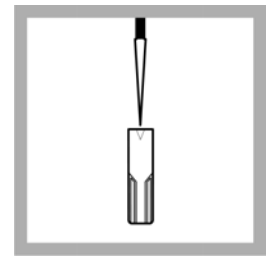
Insert an adapter if required (*Instrument-specific information*).



2. Label an Antibody Cuvette for each calibrator and each sample to be tested.



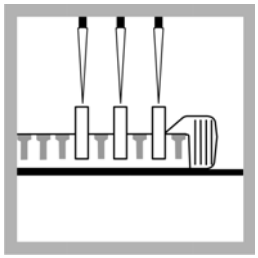
3. Insert the cuvettes into the rack snugly.



4. Pipet 0.5 mL of Diluent Solution into each Calibrator cuvette.

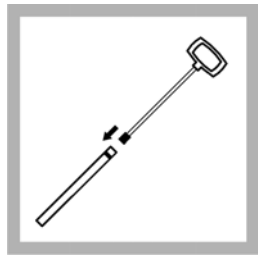
The same pipette tip can be used repeatedly for this step.

Immunoassay procedure for soil extracts and water samples (continued)



5. If testing soil: Pipet 0.5 mL of Diluent Solution into each sample cuvette.

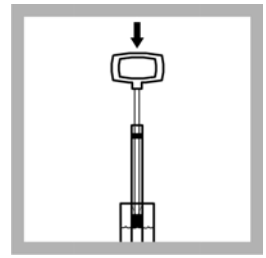
If testing water: Pipet 0.5 mL of each water sample into each sample cuvette. Use a new pipette tip for each sample.



6. Have the necessary apparatus at hand for the next four steps as they must be done without delay.

Use a Wiretrol® pipet to transfer 50 µL of each calibrator to be used into the calibrator cuvettes.

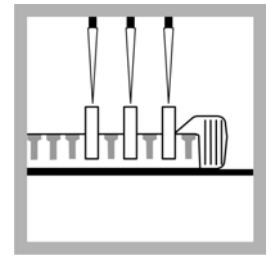
Mix the contents of the cuvettes after each addition. Use a separate capillary tube for each solution.



7. If testing soil: Use a Wiretrol pipet to transfer 50 µL of the filtered extract from step 8 of the *Soil extraction procedure* into the appropriately labeled cuvette. Use a separate capillary tube for each solution.

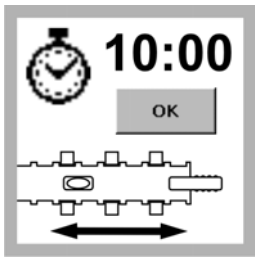
Mix the contents of the cuvettes after the addition of each sample.

If testing water: Use a Wiretrol pipet to transfer 50 µL of methanol into each sample cuvette.



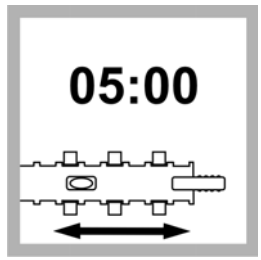
8. Immediately pipet 0.5 mL of TPH Enzyme Conjugate into each calibrator and sample cuvette.

The same pipette tip can be used repeatedly for this step.



9. Start the instrument timer for 10 minutes.

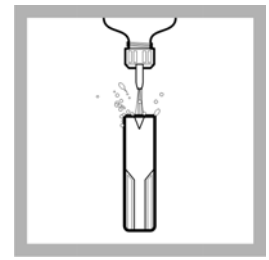
A 10-minute reaction time will begin. Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



10. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

11. At the end of the 10-minute period, discard the contents of all the cuvettes into an appropriate waste container.

The simple TPH and calibrator TPH will remain attached to the cuvette walls.



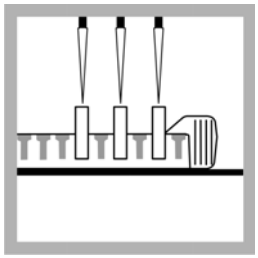
12. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Make sure that most of the water is drained from the cuvettes. Turn the cuvettes upside down and tap them lightly on a paper towel.

Immunoassay procedure for soil extracts and water samples (continued)

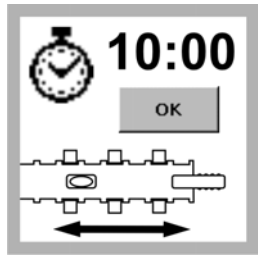
Color Development

Important Note: *Timing is critical. Follow instructions carefully.*



13. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Cuvette.

Use a new pipette tip for each cuvette.

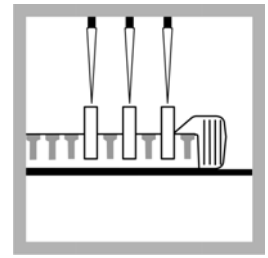


14. Start the instrument timer for 10 minutes.

A 10-minute reaction time will begin. Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

15. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same mixing technique that was used in step 14.

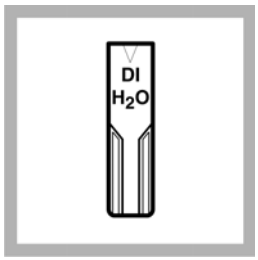
Solutions will turn blue in some or all of the cuvettes.



16. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order that was used in step 13. The same pipette tip can be used repeatedly for this step.

Mix the contents of the cuvettes for 20 seconds using the same mixing technique as in step 14.

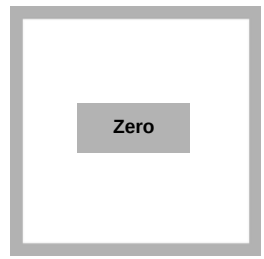
Blue solutions will turn yellow.



17. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges and fingerprints.

18. Insert the filled Zeroing Cuvette into the cell holder.

Refer to *Instrument-specific information* for cell orientation.



19. **ZERO** the instrument.

The display will show:

0.000 Abs

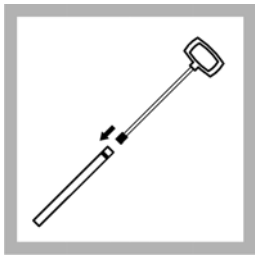
20. Insert the first calibrator into the cell holder.

READ the results. The display will give an absorbance reading. Record the results for each calibrator and sample.

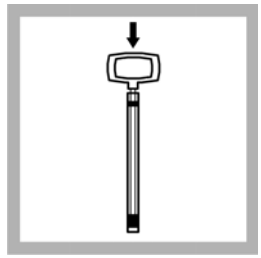
See *Interpreting and reporting results* to find the relative concentration.

Using the Wiretrol®* Pipet

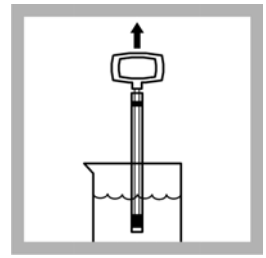
The Wiretrol Pipet can accurately measure small quantities of liquids. It consists of two parts: a Teflon®-tipped plunger and a calibrated capillary tube. The plunger can be reused. The capillary tubes must be discarded after one use.



1. Wet the orange Teflon® tip of the Wiretrol plunger in the sample and carefully insert it into the end of the capillary tube with the colored band.

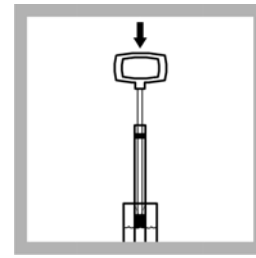


2. Push the tip to the other end of the capillary tube until it barely extends beyond the end of the capillary tube.



3. Submerge the capillary tube below the surface of the liquid to be pipetted. Slowly and smoothly draw the Wiretrol plunger up until the bottom of the plunger tips reaches the appropriate volume line.

Touch the end of the tube to the side of the vessel to release remaining drops on the capillary tube tip.



4. To discharge the pipet, place the tip of the capillary tube **below the surface of the solution** and push the Wiretrol plunger down in one smooth motion. Change capillary tubes for each calibrator and sample.

* Wiretrol is a registered trademark of Drummond Scientific.

Using the 1-cm MicroCuvette Rack

The MicroCuvette rack (Figure 1) has been designed to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

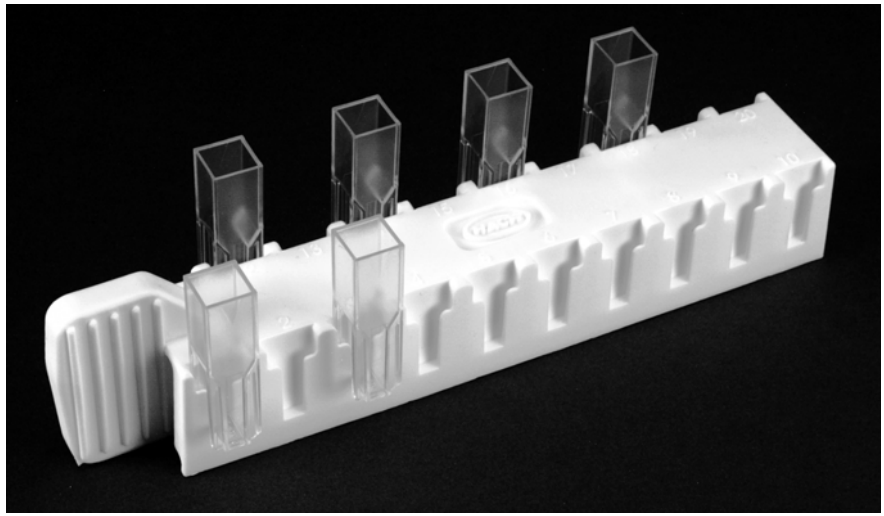


Figure 1 The 1-cm MicroCuvette Rack

Loading the Rack—The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and insert all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing—Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and reporting results

There is an inverse relationship between the concentration of TPH and the absorbance reading. In other words, the higher the absorbance reading, the lower the concentration of TPH:

- If sample reading < calibrator reading, TPH concentration in sample > calibrator reading
- If sample reading > calibrator reading, TPH concentration in sample < calibrator reading

Example:

Readings

TPH Calibrator #1: **0.480 Abs**

TPH Calibrator #2: **0.360 Abs**

Sample #1: **0.200 Abs**

Sample #2: **0.400 Abs**

Sample #3: **0.550 Abs**

Interpretation for a Soil Sample

Sample #1—The sample reading (0.200 Abs) is less than the readings for both calibrators. The concentration of TPH in the sample is greater than 50 ppm diesel fuel.

Sample #2—The sample reading (0.400 Abs) is between the readings for the TPH calibrators. The concentration of TPH in the sample is between 20 ppm and 50 ppm diesel fuel.

Sample #3—The sample reading (0.550 Abs) is greater than the readings for both calibrators. The concentration of TPH in the sample is less than 20 ppm diesel fuel.

Interpretation for a Water Sample

Sample #1—The sample reading (0.200 Abs) is less than the readings for both calibrators. The concentration of TPH in the sample is greater than 5 ppm diesel fuel.

Sample #2—The sample reading (0.400 Abs) is between the readings for the TPH calibrators. The concentration of TPH in the sample is between 2 ppm and 5 ppm diesel fuel.

Sample #3—The sample reading (0.550 Abs) is greater than the readings for both calibrators. The concentration of TPH in the sample is less than 2 ppm diesel fuel.

Storing and handling reagents

1. Wear protective gloves and eyewear.
2. When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
3. Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
4. If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The antibodies used in the TPH Test Kit react with a variety of compounds found in petroleum fuels; however, each TPH calibrator has been formulated to represent a specific concentration of diesel fuel. To use the calibrators for other TPH compounds, see *TPH compounds in soil* for soil or *TPH compounds in water* for water to select the proper TPH calibrator for the compound, sample and range you want to test.

Example:
To use the TPH calibrators for gasoline, find “Gasoline” in the first column of the*TPH compounds in soil* table or the*TPH compounds in water* table. Read across the column to find the ppm represented by each calibrator. For gasoline, calibrator #1 = 15 ppm, calibrator #2 = 35 ppm, etc.

Table 359 TPH compounds in soil

Compound	TPH calibrator #1 (ppm)	TPH calibrator #2 (ppm)	TPH calibrator #3 (ppm)	TPH calibrator #4 (ppm)
Diesel fuel	20	50	100	200
Gasoline	15	35	70	140
Kerosene	35	75	140	250
Benzene	20	45	85	160
Toluene	15	30	50	90
Ethylbenzene	5	15	35	75
m-Xylene	9	20	35	70
o-Xylene	10	20	40	80
p-Xylene	3	5	9	16
BTEX	5	15	25	45

Table 360 TPH compounds in water

Compound	TPH calibrator #1 (ppm)	TPH calibrator #2 (ppm)	TPH calibrator #3 (ppm)	TPH calibrator #4 (ppm)
Diesel fuel	2	5	10	20
Gasoline	1.5	3.5	7	14
Kerosene	3.5	7.5	14	25
Benzene	2	4.5	8.5	16
Toluene	1.5	3	5	9
Ethylbenzene	0.5	1.5	3.5	7.5
m-Xylene	0.9	2	3.5	7
o-Xylene	1	2	4	8
p-Xylene	0.3	0.5	0.9	16
BTEX	0.5	1.5	2.5	4.5

Diluting water samples

To test for TPH in water at concentrations that are higher than those shown in the *TPH compounds in water* table, dilute the sample with deionized water. Add a volume of sample from the *Dilution multipliers* table to a graduated cylinder and dilute to 50 mL with deionized water. Run the test. Multiply the calibrator levels shown in the *TPH compounds in water* table by the dilution multiplier in the *Dilution multipliers* table.

Example:
If a 0.5 mL water sample is diluted to 50 mL and tested, the calibrator levels shown in the *TPH compounds in water* table for diesel fuel would represent 200, 500, 1000 and 2000 ppm respectively.

Table 361 Dilution multipliers

mL Sample	Dilution multiplier
0.5	100
1.0	50
2.0	25
5.0	10
10.0	5
25.0	2

Interferences

Table 362 Interfering substances

Interfering substance	Interference level
Chlorine in water samples	Interferes above 2 ppm. Remove with 1 drop per 100 mL sodium thiosulfate (0.1 N).

Sample collection and storage

- Analyze the samples as soon as possible after collection.
- To store the samples, collect them in glass or Teflon® containers that have been washed with soap and water and rinsed with methanol. The container should be capped with a Teflon-lined cap. If a Teflon cap is not available, aluminum foil rinsed in methanol may be used as a substitute cap liner.
- When collecting water samples, fill the container completely (no head space) and cover the container with a tightly-sealed lid immediately after collection.
- **Soil**—Store the samples at 4 °C (40 °F) for no longer than 14 days.
- **Water**—Chill the sample in an ice bath or refrigerator to limit the loss of volatile compounds. Store samples no longer than 24 hours.

Summary of method

This method provides semi-quantitative screening for TPH based on thresholds as diesel fuel in the following concentrations:

- Soil—20, 50, 100, 200 ppm as diesel fuel
- Water—2, 5, 10, 20 ppm as diesel fuel

Immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Antibodies specific for TPH are attached to the walls of plastic cuvettes. They selectively bind and remove TPH from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and TPH compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by TPH and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of TPH in the sample. The resulting color is then compared with a calibrator to determine whether the TPH concentration in the sample is greater or less than the threshold levels. The TPH concentration is inversely proportional to the color development: the lighter the color, the higher the TPH concentration. Test results are measured at 450 nm.

Consumables and replacement items

Required reagents

Description	Unit	Catalog Number
TPH Reagent Set[1]	20 cuvettes	2774300
Deionized water	500 mL	27248

[1] Immunoassay components are manufactured by Beacon Analytical Systems, Inc.

Required apparatus

Description	Unit	Catalog Number
Caps, flip spout	2/pkg	2581802
Marker, laboratory	each	2092000
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 19700-01	1000/pkg	2185628
Rack, for 1-cm Micro Cuvettes	each	4879900
Wipes, disposable	box	2097000

Soil extraction reagents and apparatus

Description	Unit	Catalog Number
Balance, AccuLab Pocket Pro 250 B	each	2796900
Gloves, disposable, Nitrile, medium[1]	each	2550502
Pipet tips for 1970001 Ten Sette Pipet	50/pkg	2185696
Soil Scoop, 5-g, 4.25-cc	20/pkg	2657205
Soil Extraction Refill Kit, includes:	each	2775200
Dropper, LDPE, 0.5 and 1.0-mL	20/pkg	2124720
Filter and Barrel Assembly	20/pkg	2567620
Soil Extractant Solution	200 mL	2567729
Soil Sample Container	20/pkg	2592920
Weighing Boat, 8.9-cm square	20/pkg	2179020
Spatula, disposable	2/pkg	2569320
Sodium Sulfate, anhydrous	250 g	709929

[1] Other sizes are available

Optional reagents and apparatus

Description	Unit	Catalog number
Analytical balance, 80 g capacity, 0.1 mg resolution	each	2936701
Weighing papers	500/pkg	1473800
Safety goggles, vented	each	2550700
Graduated cylinder, 10 mL	each	108138
Pipet, Wiretrol®, 10–50 µL	20/pkg	2852200
Pipet, Wiretrol, 50–1000 µL	250/pkg	2568905
Sodium Thiosulfate standard solution, 0.1 N	100 mL MDB	32332

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Edition 7

Trihalomethanes

DOC316.53.01143

THM Plus™ Method **Method 10132**

(10 to 600 ppb as Chloroform) **Water Bath Method**

Scope and Application: For screening THMs in drinking water samples and Formation Potential tests.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 363 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2427606 and 2495402	Sample cell faces right
DR 5000	2427606 and 2495402	Sample cell faces user
DR 3900	2427606 and 2495402	Sample cell faces user
DR 3800, DR 2800, DR 2700	2427606 and 2495402	Sample cell faces right

Before starting the test:

Analyze the samples immediately after collection or refrigerate the samples until the analysis is complete.
If the samples were refrigerated after collection, do not warm the samples to room temperature prior to analyzing. This will minimize volatilization of the disinfection by-products (DBPs). If refrigerated samples are analyzed, heat the samples for an additional two minutes (total of seven minutes) in step 12 of the procedure.
If analyzing more than four samples, use 450 mL of water in the water bath.
THM Plus Reagent 2 **must** be at room temperature before use.
A bottle-top dispenser may be used in place of the TenSette® Pipet.
Trihalomethane compounds are extremely volatile. Immediately cap sample cells after filling with sample.
Reagent blank is stable for 1–2 hours and need not be prepared for each test.
Do not mark below the 10 mL fill line.

Collect the following items:

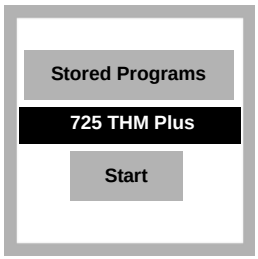
Description	Quantity
THM Plus Reagent Set	varies
Beaker, 600-mL	1
Cell Holder Assembly, TTHM	1
Evaporating Dish, 125 mm x 65 mm	2
Hot Plate, 7 x 7 inch	1
Pipet, TenSette®, 0.1–1.0 mL and tips	1
Pipet, TenSette®, 1–10 mL and tips	1
Sample Cells, 10-mL (see the *Instrument-specific information* table)	2
Wipers, disposable	varies
Ice[1]	varies

See *Consumables and replacement items* for reorder information.

[1] Depending on the temperature of the tap water, ice may be needed for the cooling baths used in steps 14 and 17.

THM Plus Method

Important Note: *Perform steps 4–9 rapidly to avoid loss of THMs from the sample. When testing more than one sample, complete steps 4–9 for one sample before going on to another. If dispensing sample with a pipette, the pipette must dispense quickly without causing aeration or back pressure.*

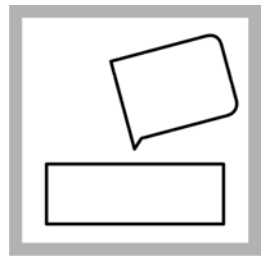


1. Select the test.

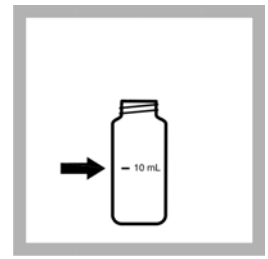
Insert an adapter if required (see the *Instrument-specific information* table).

Refer to the user manual for orientation.

2. Prepare a hot water bath by adding 500 mL of water to an evaporating dish. Put the dish on a hot plate and turn the heater on high.

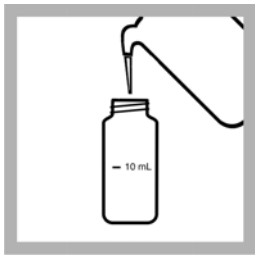


3. Prepare a cooling bath by adding 500 mL of cold (18–25 °C) tap water to a second evaporating dish. Maintain the temperature in this range.



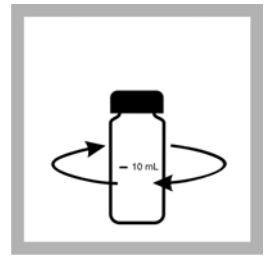
4. Prepared Sample: Fill one round sample cell to the 10-mL mark with sample. Cap and label as “sample”.

THM Plus Method (continued)



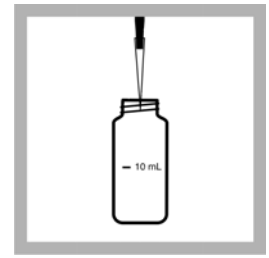
5. Blank Preparation: Fill the second sample cell with deionized water. Cap and label as “blank”.

6. Add three drops of THM Plus Reagent 1 to each cell.



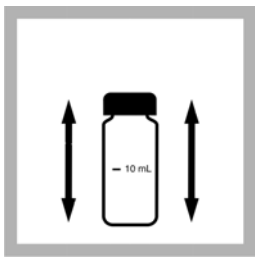
7. Cap tightly and mix gently by swirling each cell three times.

Vigorous shaking can cause loss of THMs into the sample cell headspace.



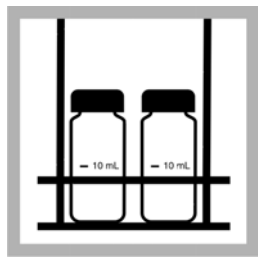
8. Use a TenSette® Pipet to add 3 mL of THM Plus Reagent 2 to each cell. Avoid excess agitation of the sample when dispensing the reagent.

The reagent is viscous and a small amount may remain on the tip after dispensing. This will not affect the results.



9. Cap tightly and mix by shaking.

Thorough mixing makes sure that all of the THM goes into the liquid and does not accumulate in the air above the sample.



10. Place the sample cells in the cell holder assembly.

11. Place the assembly in the hot water bath when the water is boiling rapidly.

Do not allow the water to rise above the white “diamond” near the top of the sample cells.

12. Start the instrument timer.

A five-minute reaction period will begin.

Heat for 7 minutes if refrigerated samples are being analyzed.

THM Plus Method (continued)

13. When the timer expires, remove the assembly and sample cells from the hot water bath. Place in the cooling bath.

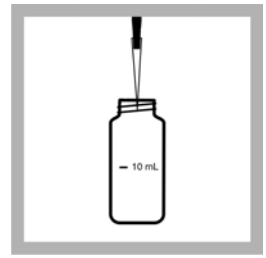
Use ice to cool the tap water if necessary.

14. Start the instrument timer.

A three-minute cooling period will begin.

When the timer expires, remove the cells from the cooling bath.

Invert each cell a few times to make sure that a uniform temperature of the sample is maintained.



15. Use a TenSette Pipet to add 1 mL of THM Plus Reagent 3 to each cell. The sample and blank will become warm.

16. Replace the cooling water with fresh, cold tap water. Place the assembly that contains the sample and blank cells into the cooling bath.

Use ice to cool the tap water if necessary.

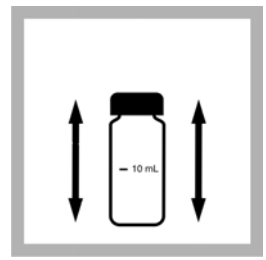
17. Start the instrument timer.

A three-minute cooling period will begin.

When the timer expires, remove the cells from the cooling bath.

The temperature of the sample should be 15–25 °C.

18. Add the contents of one THM Plus Reagent 4 Powder Pillow to the sample cell and one to the blank.



19. Cap each cell tightly and mix by shaking until all the powder dissolves.

The powder dissolves slowly. Intermittent shaking during the first five minutes of the color development period will help dissolve the reagent powder.

20. Start the instrument timer.

A 15-minute development time will begin.

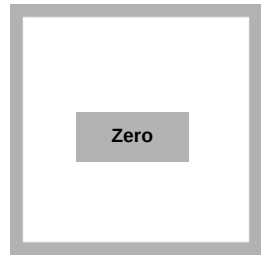
The color is stable for at least 30 minutes after the 15-minute development time.

THM Plus Method (continued)

21. After the timer expires, pour the prepared sample and prepared blank into two square sample cells.

Allow the solution to settle in the square cells for 30 seconds to enable any turbidity that may be present to settle.

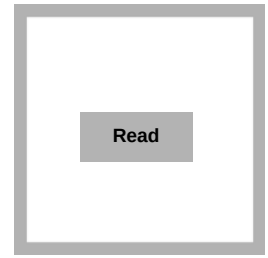
22. When the timer expires, wipe the blank and insert it into the cell holder.



23. **ZERO** the instrument.

The display will show:

0 ppb $CHCl_3$



24. Wipe the prepared sample and insert it into the cell holder.

READ the results in ppb chloroform ($CHCl_3$).

Interferences

Table 364 Interfering substances[1]

Interfering substance	Interference level
Chlorine	10 mg/L
Copper	1000 mg/L
Hardness, Ca	1000 mg/L as $CaCO_3$ May have some turbidity until Reagent 3 is added
Hardness, Mg	4000 mg/L as $CaCO_3$ May have some turbidity until Reagent 3 is added
Iron	10 mg/L
Lead	2 mg/L
Mercury	10 mg/L
Monochloramine	20 mg/L
Nickel	10 mg/L
Sodium Bisulfite	100 mg/L
EDTA	Interferes negatively at all levels

[1] The substances in the *Interfering substances* table have been tested and found to cause no interference up to the indicated levels.

Table 365 Additional disinfection by-products (DBPs) that are included in results

Compound	Effect
1,1,1-trichloro-2-propanone	Interferes positively
1,1,1-tricholoacetonitrile	Interferes positively
Chloral hydrate	Interferes positively
Dibromochloroacetic acid	Interferes positively

Table 365 Additional disinfection by-products (DBPs) that are included in results

Compound	Effect
Dichlorobromoacetic acid	Interferes positively
Tribromoacetic acid	Interferes positively
Trichloroacetic acid	Interferes positively

Sample collection, preservation and storage

- Collect samples in 40-mL glass bottles sealed with Teflon®-lined septa caps.
- Fill the bottles slowly to overflowing so that no air is included with the sample.
- Seal the bottles tightly and invert to check that no air has been trapped.
- Because trihalomethane compounds (THMs) are extremely volatile, immediate analysis yields the greatest accuracy. If the samples cannot be analyzed immediately, cool samples to 4 °C. This will slow the formation of any additional THM compounds in chlorinated samples.
- Store the samples at 4 °C in an atmosphere free of organic vapors. Samples should not be held more than 14 days. 0.1 N Sodium Thiosulfate can be used to dechlorinate samples for longer storage.
- Add 1 drop of 0.1 N Sodium Thiosulfate to dechlorinate a finished or distribution system sample collected in a 125 mL bottle.

Accuracy check

Required for accuracy check:

- THM Standard Ampule, 10 mg/L as chloroform
- Ampule breaker
- Wiretrol™ Pipet

Note: *Make sure that the chloroform is not lost to volatilization when attempting to add the chloroform to the solution. Make sure that the chloroform ampule is kept cold (can use a small ice-bath).*

Standard additions method (sample spike)

1. Open a THM Standard Ampule, 10 ppm as chloroform.
2. Use a Wiretrol Pipet to transfer 0.100 mL (100 µL) of the chloroform standard into a fresh 10 mL portion of sample.
3. Immerse the end of the pipet tip under the water and slowly dispense the chloroform.
4. Cap the sample cell immediately and swirl three times to mix.

Note: *The accuracy check methods require careful attention to technique, for it is very easy to lose the chloroform to volatilization when attempting to add it to the solution. Make sure the chloroform ampule is kept cold (may wish to use a small ice-bath)*

5. Immediately start steps 6–24 of the procedure to analyze the spiked sample.
6. The value of the spiked sample should increase 100 +/- 20 ppb over the value obtained on the original unspiked sample.
7. Calculate the % Recovery:

$$\%\ \text{Recovery} = \frac{\text{ppb THMs Spiked Sample} - \text{ppb THMs Unspiked Sample}}{\text{100 ppb THM Added}} \times 100$$

Standard solution method

1. Prepare a 99 ppb chloroform standard by pipetting 10.0 mL of organic-free water into a sample cell. Open a THM Standard Ampule, 10 ppm as chloroform. Use a Wiretrol Pipet to transfer 0.100 mL (100 µL) of the chloroform standard into the organic-free water. When adding the standard into the sample, discharge the pipet slowly at or near the bottom of the sample cell with a slight swirling motion.

   Note: *If the aliquot of the standard is discharged too quickly, the solution will form a single bubble which will rise to the top of the solution and volatilize, without being absorbed in the solution.*

2. Cap the sample cell immediately and swirl three times to mix.
3. Immediately start steps 6–24 of the procedure. Do not make up the standard in advance. Use the standard immediately upon preparation.
4. To adjust the calibration curve using the reading obtained with the 99 ppb Standard Solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**
5. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
725	66 ppb $CHCl_3$	53–79 ppb $CHCl_3$	19 ppb $CHCl_3$

Summary of method

The THM Plus method reacts with the trihalogenated disinfection by-products formed as the result of the disinfection of drinking water with chlorine in the presence of naturally occurring organic materials. These disinfection by-products (DBPs) may be produced in the treatment plant or the distribution system as long as the water is in contact with free chlorine residual. The formation of the DBPs is influenced by chlorine contact time, chlorine dose and residual, temperature, pH, precursor concentration, and bromide concentration.

The predominant DBPs formed by the chlorination of drinking water are the trihalomethanes or THMs. The four trihalogenated compounds that form are chloroform, bromoform, dichlorobromomethane, and dibromochloromethane. These four compounds comprise the Total Trihalomethanes (TTHMs) group which is regulated under the Safe Drinking Water Act. The combined concentration of the TTHMs, is regulated in drinking water samples. Other DBPs that may be present and react under the conditions of the THM Plus method are listed in Interferences.

In the THM Plus method, THM compounds present in the sample react with N, N,-diethylnicotinamide under heated alkaline conditions to form a dialdehyde intermediate. The sample is then cooled and acidified to pH 2.5. The dialdehyde intermediate formed is then reacted with 7-amino-1,3 napthalene disulfonic acid to form a colored Schiff base. The color formed is directly proportional to the total amount of THM compounds present in the sample. Test results are measured at 515 nm and reported as ppb chloroform.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Reagent Set (50 tests[1]), includes:			2790800
THM Plus™ Reagent 1	6 drops	15 mL	2753929
THM Plus™ Reagent 2	6 mL	330 mL	2754048
THM Plus™ Reagent 3	2 mL	110 mL	2754142
THM Plus™ Reagent 4	2 pillows	100 pillows	2756699

[1] Fifty tests equals 25 samples and 25 individual blanks. Additional tests can be obtained when multiple samples are run using a single blank.

Required apparatus

Description	Quantity	Unit	Catalog number
Beaker, 600-mL	1	each	50052
Cell Holder Assembly, TTHM	1	each	4788000
Evaporating Dish, 125 mm x 65 mm	2	each	2764700
Hot Plate, 7 x 7 in., 115 VAC, digital	1	each	2881600
Hot Plate, 7 x 7 in., 230 VAC, digital	1	each	2881602
Pipet, TenSette®, 0.1–1.0 mL	1	each	1970001

Required apparatus

Description	Quantity	Unit	Catalog number
Pipet Tips for TenSette Pipet 19700-01	varies	50/pkg	2185696
Pipet, TenSette®, 1–10 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	varies	50/pkg	2558996
Wipers, disposable	varies	280/pkg	2097000

Recommended standards

Description	Unit	Catalog number
Chloroform, 10-ppm ampule	7/pkg	2756707
Water, Reagent, Organic-free	500 mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet, filler, safety bulb	each	1465100
Pipet, volumetric, class A, 10 mL	each	1451538
Pipettes, Wiretrol™, 50–100 µL	250/pkg	2568905
Vials, glass, 40-mL, with Septa cap	5/pkg	2794005
Sodium thiosulfate standard solution, 0.1 N	100 mL	323-32

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Edition 7

Trihalomethane Formation Potential (THMFP)

DOC316.53.01147

Method 10224[1]

THM Plus

Scope and Application: For determining the potential of potable source waters to form trihalomethanes and other disinfection by-products when under the influence of direct chlorination. For evaluating water treatment processes, water sources or for predicting THM concentrations in a distribution system. For running Simulated Distribution System Trihalomethanes (SDS-THM) studies.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater, Section 5710*

Test preparation

Before starting the test:

Develop a formation potential test plan. See *Trihalomethane formation potential test plan* on page 1216.
Precondition sample containers, test bottles and glassware to be chlorine demand free. See *Treating analysis labware* on page 1217.
Bring sample to the temperature prescribed in the formation potential plan before preparing the samples.

Collect the following items:

Description	Quantity
DPD Free Chlorine Reagent	Varies
Chlorine Dosing Solution Ampules	Varies
THM Plus Reagent	Varies
Sample Bottles/Caps	6
Bottle Labels	6
pH Meter	1
Thermometer	1
Pipet, TenSette®, 0.1 to 1.0 mL and tips	1
Sample Cells, 10 mL, with caps	2
Stirrer/Hotplate	1
Stir Bar Magnet	1
Cell Holder Assembly and Evaporating Dish (water bath)	1
Spectrophotometer	1

THM Plus

1. Complete a *Trihalomethane formation potential test plan* (page 1216).

Measure and record the temperature and pH of the sample water to be tested.

If the test plan temperature is different from the collected sample, condition the sample to the test plan temperature before continuing the test.

2. Prepare six chlorine demand-free bottles. Rinse each bottle with sample and fill each 118-mL bottle (bottles contain 125 mL when filled to overflowing) with approximately 100 mL of the sample to be tested. Label the bottles 1 through 6.

If the study is run with fixed pH, add the pH buffer to the sample before filling the bottles.

3. Do not handle the stir bar with fingers.

Use tweezers or tongs to insert a stir bar magnet into each bottle. Set Bottle #1 on a stir plate and stir gently. A small vortex should be visible on the surface of the liquid.

4. Open a Chlorine Dosing Solution Ampule. Using a TenSette Pipet, add 0.2 mL of the chlorine solution to Bottle #1 while stirring. Immerse the end of the pipet tip under the water to dispense the chlorine.

Mixing while adding the chlorine is imperative to avoid highly localized areas of chlorine concentration.

5. Turn off the stirrer and fill the bottle until it is overflowing with sample. Cap in a manner to avoid trapping any air bubbles and invert to mix. Put the sample bottle in the dark or wrap with foil.

Each 0.2 mL of Dosing Solution added will add approximately 2.0 mg/L Cl_2 to the sample.

6. Calculate the actual amount of chlorine added (*Equation 1* on page 1215).

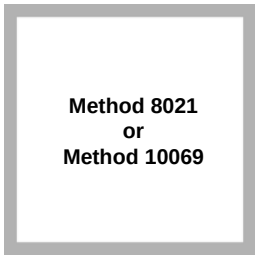
Repeat steps 4–5

7. Repeat Steps 4–5 for bottles 2 through 6. Increase the amount of chlorine added in increments of 0.2 mL (Table 366).

The amounts added may be increased or decreased based on the expected chlorine demand of the sample water and chlorine contact time.

8. Incubate or refrigerate the bottles at the temperature and contact time specified in the test plan.

THM Plus



9. After the prescribed chlorine contact time is completed, analyze the samples for residual Free Chlorine using DPD Free Chlorine Reagent (Method 8021 or Method 10069). Follow the chlorine procedure supplied with the spectrophotometer being used.

10. Select the sample bottle or bottles having the prescribed chlorine residual from the test plan and analyze for THMs using the THM Plus method (Method 10132)[1], [2].

Follow the procedure supplied with the spectrophotometer being used. Results are µg/L (ppb) chloroform.

[1] Recheck the pH of the sample remaining in the analyzed bottle to check for pH shifts that may occur in low alkalinity waters.

[2] The selected bottle or bottles to be analyzed for THMs should be analyzed as soon as possible, especially bottles that have had sample removed when testing for chlorine residual. If this is not possible, add 1 drop of 0.1 N sodium thiosulfate and store the samples at 4 °C for up to 14 days.

Chlorine addition calculation

Use *Equation 1* to calculate the concentration of the chlorine added in step 6.

Equation 1

$$\text{mg/L } Cl_2 = \frac{0.2 \text{ mL } \langle \text{volume of standard added} \rangle \times \text{ampule certificate value } \langle \text{ mg/L } Cl_2 \rangle}{125 \text{ mL}}$$

Example:

$$\text{mg/L } Cl_2 = \frac{0.2 \text{ mL} \times \text{certificate value of } 1250 \text{ mg/L } Cl_2}{125 \text{ mL}}$$

mg/L Chlorine = **2.0**

Incremental reagent addition

Table 366 defines the incremental addition of chlorine dosing solution.

Table 366 Incremental addition of a 1250 mg/L Cl_2 dosing solution

Bottle #	Cl_2 dosing solution added (mL)	Increases sample concentration (in mg/L Cl_2) by
1	0.2	2.0
2	0.4	4.0
3	0.6	6.0
4	0.8	8.0
5	1.0	10.0
6	1.2	12.0

Trihalomethane formation potential test plan

Formation potential tests are run to determine the potential of a water to form THMs or other disinfection by-products under a standard or a user-specified set of reaction conditions. The goals of the test plan should be well defined and the reaction conditions clearly documented to support the goals of the plan.

Formation potential plans are often structured to have a fixed sample pH, sample temperature, chlorine dose, chlorine contact time and a desired chlorine residual after the contact time. Formation plans with fixed pH, temperature, chlorine concentration and chlorine contact times are designed to compare the variability of various waters while holding the variables responsible for THM formation constant. This allows the evaluation of different source waters and allows the results to be duplicated at different utilities or by different analysts under similar reaction conditions. Some standard or fixed reaction condition plans available in the published literature or in Standard Methods are listed here as examples.

Reaction conditions such as the standard 7-day reaction (incubation) period at pH 7.0 ± 0.2 with phosphate buffer at 25 ± 2 °C with a remaining chlorine residual between 3.0 and 5.0 mg/L are not intended to simulate water treatment processes. These conditions are most useful for estimating the concentration of THM precursors in the raw water or for measuring the effectiveness of a treatment change under fixed standard conditions. A second standard method using a 3-day reaction time has also been used. Additionally, a Uniform Formation Conditions (UFC) test is available that uses a contact time of 24 ± 1 hour at 20.0 ± 1.0 °C, a borate buffer at pH 8.0 ± 0.2 with a target free chlorine residual of 1.0 ± 0.4 mg/L after 24 hours as the sample bottle to be measured for THMs. The Formation Potential Plan can also be constructed to develop a Simulated Distribution System Trihalomethanes (SDS-THM) study. The goal of this plan is to use the existing treated water pH with variable chlorine doses and contact times that simulate the chlorine levels and water age found in a distribution system. This will estimate the THM formation occurring in the distribution system.

Another important use of Formation Potential Test plans is to use a single water source and vary temperature, pH or chlorine contact times to meet specific treatment optimization goals. It is again important to document all reaction conditions used in the test plan.

Examples of additional formation potential studies are:

- Formation potential studies on settled waters from jar tests to evaluate the effectiveness of coagulant dosage on removing organics responsible for THM formation.
- Formation potential studies based on chlorine dosage rates and location of chlorine application.
- Formation potential studies on the rate of THM formation at variable locations within the treatment stream.

- Formation potential studies to correlate THM levels to UV-254, TOC or SUVA values.
- Formation potential studies to study the effects of using alternative oxidants.

Formation potential procedure modification

Use the following guidelines whenever modifications are made:

- Make smaller chlorine concentration additions by using a larger sample size or smaller chlorine additions. A 237-mL bottle (contains 250 mL when filled to overflowing) is available for low chlorine demand applications. Each 0.1 mL of chlorine standard added will add approximately 0.5 mg/L of chlorine. Substitute 250 mL for 125 mL in *Equation 1*. A lower concentration Chlorine Standard Solution, 50–75 mg/L as Cl_2 is available for testing low organic waters.
- High organic waters require larger additions of chlorine. Use 0.5 mL, 1.0 mL, 1.5 mL, etc., to spike the bottles in steps 4 and 7 in the test procedure.
- Wrap sample bottles made of clear colorless glass in foil to protect the sample from light, or be kept in the dark during the contact time.
- Sample pH can be modified or standardized by adding a fixed amount of a pH buffer solution to each bottle. Prepare a reagent blank bottle using organic free water. Add the same amount of buffer to this blank and carry the blank through the procedure. This will check the formation potential (if any) that was added by the buffer. Subtract the formation potential of the blank from the sample THM values.

Sampling and storage

Collect samples in glass bottles sealed with TFE-lined screw caps. Use only freshly collected samples and process immediately. If this is not possible, store samples a 4 °C and analyze as soon as possible. Significant sample degradation can occur in unpreserved samples within 24 hours. Add one drop of thiosulfate solution per 125 mL sample bottle if the samples are chlorinated and cannot be analyzed immediately.

Treating analysis labware

Glassware used in this test must be chlorine demand-free. Treat all glassware with a dilute solution of chlorine bleach prepared by adding 0.5 mL of commercial bleach to 1 liter of water. Alternatively, the sample bottles may be treated by adding 2.0 mL of the Chlorine Dosing Solution to each 125-mL bottle and filling to overflowing with deionized water. Soak glassware in this solution for at least one hour. After soaking, rinse the glassware with copious amounts of chlorine demand-free water before filling with sample.

Summary of method

Organic matter present in drinking water source waters reacts with chlorine to form chlorinated organic species, some of which may be trihalomethanes or other regulated disinfection by-products. The potential of various source or treated waters to form disinfection by-products can be determined by adding chlorine and controlling dosage rate, pH, temperature and contact time. The THMs formed under these user-defined conditions are determined using the THM Plus method.

Consumables and replacement items

Required reagents

Description	Catalog number
Chlorine Dosing Solution Ampules, 1190–1310 mg/L as Cl_2, 10-mL ampules, 16/pkg	2504810
Chloroform, 10 ppm ampule, 7/pkg	2756707
DPD Free Chlorine Reagent Powder Pillows, 25 mL	1407099
THM Plus Reagent Set	2790800
Water, Organic Free, 500 mL	2641549

Required apparatus

Description	Catalog number
Bottles, Amber Glass, 118 mL, 6/pkg	714424
Caps, Black, PP Teflon liner, 12/pkg	2401812
Cell Holder assembly, TTHM	4788000
Evaporating Dish, 125 mm x 65 mm	2764700
Hot Plate/Stirrer, 7 x 7 inch, 110 V	2881600
Sample cells, 10 mL w/caps	2427606
Stir Bar, Teflon-coated, 2.22 x 0.48 cm	4531500

Optional reagents and apparatus

Description	Catalog number
Ampule Breaker, for Voluette® Ampules	2196800
Bottles, Amber Glass, 237 mL, 6/pkg	714441
Buffer Powder Pillows, pH 6.86, 15/pkg	1409895
Buffer Powder Pillows, pH 8.00, 15/pkg	1407995
Buffer Powder Pillows, pH 8.3, 25/pkg	89868
Buffer Solution, pH 7.0, Demand Free, 500 mL	2155353
Caps, for 714441 Bottles, 6/pkg	2166706
Chlorine Standard Solution Ampule, 50–75 mg/L as Cl_2, 10 mL, 16/pkg	1426810
DPD Free Chlorine AccuVacs®, 25/pkg	2502025
DPD Free Chlorine Reagent Powder Pillows, 10 mL	2105599
DPD Free Chlorine Swiftest Dispenser w/reagents	2802300
Free Chlorine, Swiftest Dispenser Reagent (refill)	2105560

Optional reagents and apparatus (continued)

Description	Catalog number
Incubator, Model 205, 110 V, 0 to 40 °C	2616200
Labels, PolyPaper, 1.5 x 3 " 120/pkg	2091502
Pipettes, Wiretrol, 50–100 µL	2568905
Sens***ion*** 2 Portable pH/ISE Meter w/electrode	5172510
Sodium Hydroxide Standard Solution, 0.100N, 500 mL	19153
Sodium Thiosulfate Solution, 0.1 N, 100 mL	32332
Standard Methods Handbook	2270800
Sulfuric Acid Standard Solution, 0.100 N, 500 mL	20253
Thermometer, Double Scale, –20 to 110 °C (0 to 230 °F)	2095911
TenSette Pipet, 0.1 to 1.0 mL	1970001
Tips for TenSette Pipet, 0.1 to 1.0 mL, 50/pkg	2185696
Tweezers	1428200

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Edition 7

Volatile Acids

DOC316.53.01144

Esterification Method[1] **Method 8196**

(27 to 2800 mg/L) **Reagent solution**

Scope and Application: For digestor sludges.

[1] Adapted from *The Analyst, 87, 949 (1962).*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 367 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2401906 and 2495402	Fill line faces right
DR 5000	2401906 and 2495402	Fill line faces user
DR 3900	2401906 and 2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2401906 and 2495402	Fill line faces right

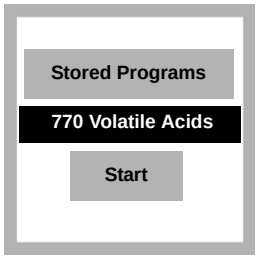
Collect the following items:

Description	Quantity
Centrifuge	1
Centrifuge Tubes and Caps	2
Cylinder, 10-mL graduated	1
Ethylene Glycol	3 mL
Ferric Chloride-Sulfuric Acid Solution	20 mL
Funnel and Filter Paper	—
Hot Plate	1
Hydroxylamine Hydrochloride Solution, 100-g/L	1 mL
Pipet Filler	1
Pipet, 2 mL[1]	1
Pipet, volumetric, Class A, 0.50-mL[1]	1
Pipet, volumetric, Class A,10-mL[1]	1
Sample Cells, 10-20-25 mL	2
Sodium Hydroxide Standard Solution, 4.5 N	4 mL
Sulfuric Acid Standard Solution, 19.2 N	0.4 mL
Water Bath and Rack	1
Water, deionized	20.5 mL

See *Consumables and replacement items* for reorder information.

[1] The TenSette Pipet can be used in place of individual pipets in this procedure.

Esterification Method



1. Select the test.

Insert an adapter if required (*Instrument-specific information*).

Refer to the user manual for orientation.

2. Blank Preparation: Pipet 0.5 mL of deionized water into a dry 25-mL sample cell.

3. Filter or centrifuge 10 mL of sample. Centrifuging is faster than filtration.

4. Prepared Sample: Pipet 0.5 mL of the filtrate or supernatant into a second dry 25-mL sample cell.

5. Pipet 1.5 mL of ethylene glycol into each sample cell. Swirl to mix.

6. Pipet 0.2 mL of 19.2 N Sulfuric Acid Standard Solution into each cell. Swirl to mix.

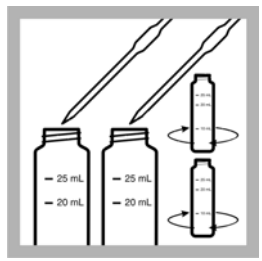
7. Insert both cells into a boiling water bath.

Alternatively, the cells may be boiled in a 500-mL beaker.

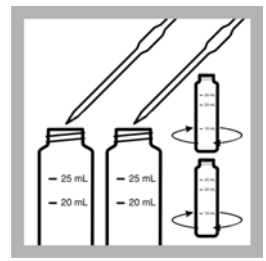
8. Start the instrument timer.

A three-minute reaction period will begin.

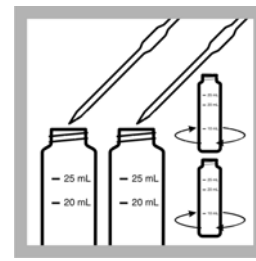
9. When the timer expires, cool the solutions to 25 °C (until the cell feels cold) with cool water bath.



10. Using a pipet filler, pipet 0.5 mL of Hydroxylamine Hydrochloride Solution into each cell. Swirl to mix.

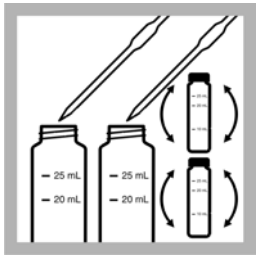


11. Using a pipet filler, pipet 2.0 mL of 4.5 N Sodium Hydroxide Standard Solution into each cell. Swirl to mix.



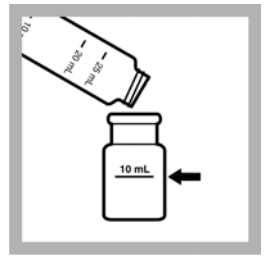
12. Add 10 mL of Ferric Chloride Sulfuric Acid Solution to each cell. Swirl to mix.

Esterification Method (continued)



13. Add 10 mL of deionized water to each cell. Cap and invert to mix.

14. Transfer 10 mL of the blank solution from the round 25-mL cell to a clean dry square sample cell.

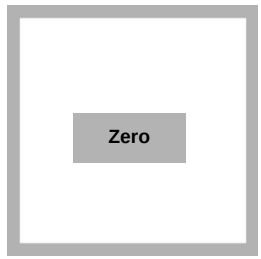


15. Transfer 10-mL of the sample solution from the round 25-mL cell to a clean dry square sample cell.

16. Immediately start the instrument timer.

Another three-minute reaction period will begin. During this time, complete steps 17 and 18.

17. Blot each sample cell dry. Immediately insert the blank into the cell holder.

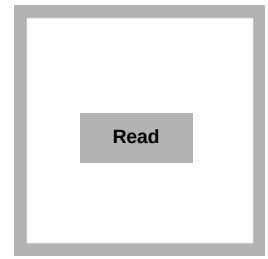


18. **ZERO** the instrument.

The display will show:

0 mg/L HOAC

19. Wipe the prepared sample and insert it into the cell holder.



20. **READ** the results in mg/L HOAC.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Analyze as soon as possible after collection.
- Samples can be stored for up to 24 hours by cooling to 4 °C (40 °F) or below.
- Warm samples to room temperature before analysis.

Accuracy check

Required for accuracy check:

- Volatile Acid Voluette® Ampule Standard, 62,500-mg/L as acetic acid
- Ampule breaker
- TenSette Pipets and tips
- Three 25-mL mixing cylinders
- 500-mL volumetric flask, Class A

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu **OPTIONS>MORE>STANDARD ADDITIONS**. Default values for standard concentration, sample volume, and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
3. Open one Voluette ampule standard.
4. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL, and 0.3 mL of standard to three 25-mL portions of fresh sample.
5. Follow the test procedure for each of the spiked samples starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
6. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

1. Prepare a 500-mg/L volatile acid standard solution as follows. Pipet 4.00 mL of Volatile Acid Standard Solution, 62,500-mg/L, into a 500-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily.
2. Use the 500-mg/L Volatile Acid Standard Solution in place of the sample. Follow the volatile acid test procedure.
3. To adjust the calibration curve using the reading obtained with the 500-mg/L standard solution, navigate to Standard Adjust in the software **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
770	1350 mg/L as acetic acid (HOAC)	1218–1482 mg/L HOAC	27 mg/L as HOAC

Summary of method

The volatile acids test is designed specifically for determining volatile acids in digestor sludges. The method is based on esterification of the carboxylic acids present in the sample and subsequent determination of the esters by the ferric hydroxamate reaction. All volatile acids present are reported as their equivalent mg/L as acetic acid. Test results are measured at 495 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Volatile Acid Reagent Set (90 tests), includes:			2244700
(1) Ethylene Glycol	3 mL	1000 mL	203953
(2) Ferric Chloride-Sulfuric Acid Solution	20 mL	1000 mL	204253
(1) Hydroxylamine Hydrochloride Solution, 100-g/L	1 mL	100 mL	81842
(1) Sodium Hydroxide Standard Solution, 4.5 N	4 mL	1000 mL	204053
(1) Sulfuric Acid Standard Solution, 19.2 N	0.4 mL	1000 mL	203832
Water, deionized	20.5 mL	4 L	27256

Required apparatus

Description	Quantity	Unit	Catalog number
Centrifuge, 115 VAC, 60 Hz.	1	each	2676500
Centrifuge Tubes, 15-mL	2	10/pkg	2278739
Centrifuge Tube Caps	2	20/pkg	2585220
Cylinder, graduated, 10-mL	1	each	50838
Filter Paper, folded, 12.5-cm	1	100/pkg	189457
Funnel, poly, 65-mm	1	each	108367
Hot Plate, 7-inch digital, 120 VAC	1	each	2881500
Hot Plate, 7-inch digital, 240 VAC	1	each	2881502
Pipet Filler, safety bulb	1	each	1465100
Pipet, serological, 2-mL	1	each	53236
Pipet, volumetric, Class A, 0.50-mL	1	each	1451534
Pipet, volumetric, Class A, 10.00-mL	1	each	1451538
Cell holder assembly	1	each	4788000
Evaporating dish, 125 mm x 65 mm	1	each	2764700
Sample cell, 10 mL square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Volatile Acids Standard Solution, 10-mL Voluette® Ampule, 62,500-mg/L as HOAC	16/pkg	14270-10
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing, 25-mL	each	189640
Water Bath and Rack	each	195555
Pipet, TenSette, 1.0 to 10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010	250/pkg	2199725
Pipet Tips, for TenSette Pipet 1970010	50/pkg	2199796
Pipet, TenSette, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696

Optional reagents and apparatus

Description	Unit	Catalog number
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Volumetric flask, Class A, 500 mL	each	1457449
Finger cots	2/pkg	1464702

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Edition 7

Volatile Acids

DOC316.53.001182

Sodium Hydroxide Method

Method 8218

100 to 2400 mg/L CH_3COOH

Digital Titrator

Scope and Application: For wastewater

Test preparation

Before starting the test:

Use the TitraStir apparatus for best results.
Distill the sample according to the *Volatile Acids Procedure, Sample Distillation* instructions in the General Purpose Distillation Apparatus Set or use the distillation procedure described in *Standard Methods for the Examination of Water and Wastewater.*
The final result has been adjusted to give the correct answer based on a 70% correction factor. For higher recoveries, use the esterification method.

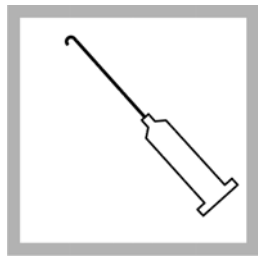
Collect the following items:

Description	Quantity
Volatile Acid Reagent Set	1
Deionized Water	varies
Digital Titrator	1
Erlenmeyer Flask, 250-mL	1
Graduated Cylinder	1

See *Consumables and replacement items* for reorder information.

Sodium Hydroxide Method

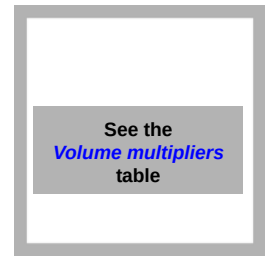
1. Collect 150 mL of distillate.



2. Insert a clean delivery tube into a 0.9274 N Sodium Hydroxide titration cartridge.

Attach the cartridge to the titrator body.

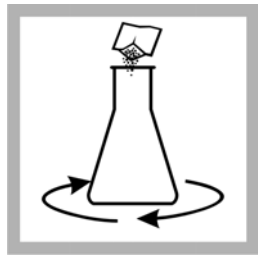
3. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.



4. Select the distillate volume that corresponds to the expected volatile acids concentration in acetic acid from the *Volume multipliers* table.

Sodium Hydroxide Method (continued)

5. Using a graduated cylinder, transfer the distillate volume into a clean, 250-mL Erlenmeyer flask and dilute to approximately the 150-mL mark with deionized water.



6. Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix.

7. Place the delivery tube tip into the solution and swirl while titrating with sodium hydroxide until a light pink color appears. Record the number of digits required.

8. Calculate:
Digits Required x
Digits Multiplier =
mg/L Volatile Acids
(as acetic acid, CH_3COOH)

Table 368 Volume multipliers

Range (mg/L as CH_3COOH)	Volume (mL)	Digit Multiplier
100–400	150	1
200–800	75	2
600–2400	25	6

Summary of method

A sample acidified with sulfuric acid is distilled and the distillate is then titrated to the phenolphthalein end point with sodium hydroxide standard.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Volatile Acid Reagent Set, includes:		2460200
Phenolphthalein Indicator Powder Pillows	100/pkg	94299
Sodium Hydroxide Titration Cartridge, 0.9274 N	each	1484201
Water, deionized	4 L	27256

Required apparatus

Description	Unit	Catalog number
Cylinder, graduated, 250-mL	each	50846
Digital Titrator	each	1690001
Flask, Erlenmeyer, 250-mL	each	50546
Cylinder, Graduated 100 mL, TD White	each	50842
Cylinder Graduated, 25 mL TD White	each	50840
Delivery Tube, 180° hook	5/pkg	1720500

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Edition 7

Volatile Acids

DOC316.53.01163

Buret Titration Method[1]

Method 8291

100 to 2400 mg/L as CH_3COOH

Buret Titration

Scope and Application: For water, wastewater and seawater.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

Before starting the test:

The sample must be distilled before this test can be started. Follow the distillation procedure that is given in the distillation manual or the distillation procedure that is given in *Standard Methods for the Examination of Water and Wastewater.*

This method recovers approximately 65 to 95% of the volatile acids in the sample, but 70% is the accepted correction factor.

The final result has been adjusted to give the correct answer based on a 70% correction factor. For higher recoveries, use the esterification method.

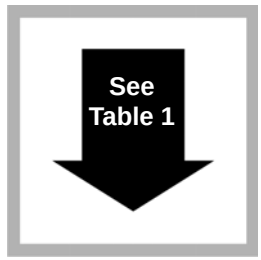
Collect the following items:

Description	Quantity
Phenolphthalein Indicator Powder Pillow	1
Sodium Hydroxide Standard Solution, 0.100 N	1 bottle
Buret, Class A, 50-mL, with support stand	1
Erlenmeyer flask, 250-mL	1
Graduated cylinder	1

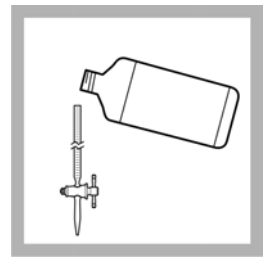
See *Consumables and replacement items* for reorder information.

Buret titration

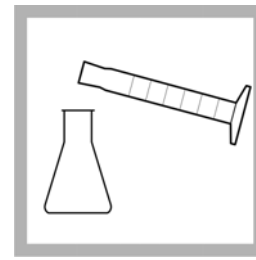
1. Distill the sample and collect 150 mL of distillate. Fully mix the distillate.



2. Select a sample volume from the *Range-specific information*.



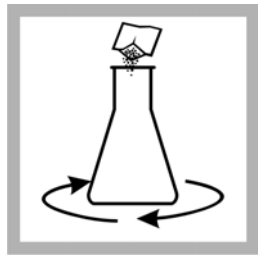
3. Fill a 50-mL buret to the zero mark with 0.100 N Sodium Hydroxide Standard Solution.



4. Use a graduated cylinder to measure the selected volume of distillate. Add the sample to a 250-mL Erlenmeyer flask.

Buret titration (continued)

5. If the sample volume is less than 150 mL, dilute to approximately 150 mL with deionized water.



6. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix.

7. Titrate the sample while swirling the flask until a light pink color forms and persists for 30 seconds.

8. Use the multiplier in the *Range-specific information* to calculate the concentration:

mL titrant x 86 x multiplier = mg/L as CH_3COOH

Example: 25 mL of sample was titrated and 3 mL of titrant was used to reach the endpoint. The concentration is 3 x 86 x 6 = 1550 mg/L CH_3COOH

Table 369 Range-specific information

Range (mg/L as CH_3COOH)	Sample volume (mL)	Multiplier
100–400	150	1
200–800	75	2
600–2400	25	6

Sample collection, preservation and storage

Collect samples in clean plastic or glass bottles. Analyze the sample as soon as possible after collection. Samples can be stored for up to 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before starting the test.

Summary of method

A sample acidified with sulfuric acid is distilled and the distillate titrated to the phenolphthalein end point with sodium hydroxide. The volume of titrant that is necessary to reach the end point is proportional to the volatile acids concentration. The results are in mg/L as acetic acid (CH_3COOH).

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
Sodium Hydroxide Standard Solution, 0.100 N	varies	1 L	19153

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Buret, Class A, 50-mL	1	each	2636541
Buret Clamp, double	1	each	32800
Flask, Erlenmeyer, graduated, 250-mL	1	each	50546
Graduated cylinder—select one or more based on range:			
Cylinder, graduated, 25-mL	1	each	50840
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Cylinder, graduated, 250-mL			50846
Support Stand	1	each	56300
Bottles, sampling, poly, 250-mL	1	each	2087076

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Edition 7

Zinc

DOC316.53.01145

USEPA[1] Zincon Method[2] **Method 8009**

(0.01 to 3.00 mg/L) **Powder Pillows**

Scope and Application: For water and wastewater. Digestion is required for a total zinc analysis (see *Digestion*).

[1] USEPA approved for wastewater analyses 3500 Zn B: Federal Register, 45(105) 36166 (May 29, 1980).

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays information that may vary from instrument to instrument. Select the spectrophotometer from the instrument column on the left. Read across to find the corresponding sample cells and adapters required to perform this test on the spectrophotometer.

Table 370 Instrument-specific information

Instrument	Powder pillows	
	Sample cell	Cell orientation
DR 6000	2495402	Fill line faces right
DR 5000	2495402	Fill line faces user
DR 3900	2495402	Fill line faces user
DR 3800, DR 2800, DR 2700	2495402	Fill line faces right

Before starting the test:

Use only glass-stoppered mixing cylinders in this procedure.
Wash glassware with 1:1 HCl[1] and rinse with deionized water before use.
Use a plastic dropper in step **6** of this procedure. Droppers with rubber bulbs may contaminate the reagent.
ZincoVer® 5 reagent contains potassium cyanide. Cyanide solutions are regulated as hazardous waste by the Federal RCRA. Cyanide should be collected for disposal as a reactive (D003) waste. Be sure that cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. Refer to the current MSDS for handling and disposal information.
The Pour-Thru Cell cannot be used with this test.

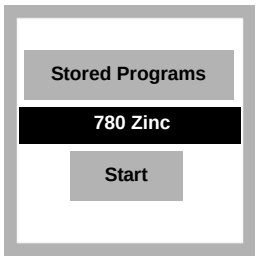
[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
Cyclohexanone	0.5 mL
ZincoVer 5 Reagent Powder Pillow	1
Cylinder, graduated mixing, 25-mL	1

See *Consumables and replacement items* for reorder information.

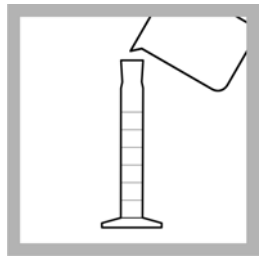
Zincon method



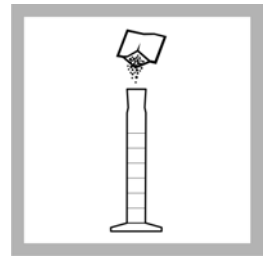
1. Select the test.

Insert an adapter if required (the *Instrument-specific information* table).

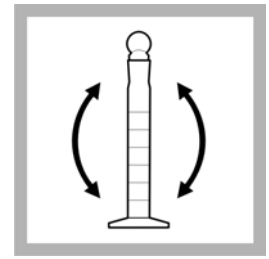
Refer to the user manual for orientation.



2. Fill a 25-mL graduated mixing cylinder with 20 mL of sample.



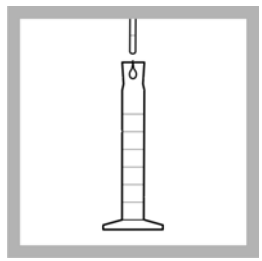
3. Add the contents of one ZincoVer 5 Reagent Powder Pillow to the mixing cylinder. Stopper.



4. Invert several times to dissolve the powder completely. Inconsistent readings may result if all the particles are not dissolved.

The sample should be orange. If the sample is brown or blue, the zinc concentration is too high or an interfering metal is present. Dilute the sample and repeat the test.

5. Blank preparation: Pour 10 mL of the solution into a sample cell.



6. Prepared sample: Use a plastic dropper to add 0.5 mL of cyclohexanone to the remaining solution in the mixing cylinder.

7. Start the instrument timer. A 30-second reaction period will begin.

During the reaction period, stopper the mixing cylinder and vigorously shake the prepared sample.

The sample will be reddish-orange, brown, or blue, depending on the zinc concentration.

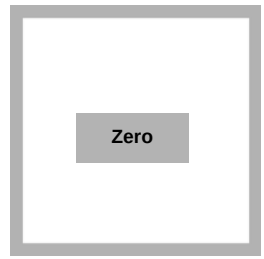
8. Start the instrument timer.

A three-minute reaction period will begin. During this reaction period, complete step **9**.

Zincon method

9. Pour the prepared sample solution from the mixing cylinder into a second sample cell.

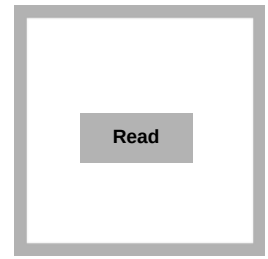
10. When the timer expires, wipe the blank and insert it into the cell holder.



11. **ZERO** the instrument.

The display will show:

0.00 mg/L Zn



12. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L Zn.

Interferences

Table 371 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 6 mg/L
Cadmium	Greater than 0.5 mg/L
Copper	Greater than 5 mg/L
Iron (ferric)	Greater than 7 mg/L
Manganese	Greater than 5 mg/L
Nickel	Greater than 5 mg/L
Organic Material	Large amounts may interfere. Pretreat the sample with a mild digestion.
Highly buffered or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment. Adjust pH to 4–5.
Amino-tri(methylene phosphonic acid) (AMP)	Samples containing AMP cause a negative interference. Digest the sample to eliminate this interference (follow the total phosphorus hot plate digestion, Method 8190). ***Important Note:*** *Be sure to adjust the pH of the sample after the digestion to pH 4–5 with sodium hydroxide before the zinc analysis. Correct the pH level for volume changes.*

Sample collection, preservation and storage

Collect samples in acid-cleaned plastic or glass bottles. If prompt analysis is impossible, preserve the sample by adjusting to pH 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to 4–5 with 5.0 N Sodium Hydroxide. Do not exceed pH 5 as zinc may precipitate. Correct the test result for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Zinc Voluette® Ampule Standard, 25 mg/L Zn
- Ampule breaker
- TenSette Pipet 0.1 - 1.0 mL and tips
- 25-mL mixing cylinders

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select **OPTIONS>MORE>STANDARD ADDITIONS** from the instrument menu.
3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in the top row.
4. Open one Voluette ampule standard.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL, and 0.3 mL of standard to three 20-mL portions of fresh sample.
6. Follow the test procedure for each of the spiked samples starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 100 mg/L zinc standard solution
- 10.00 mL Class A pipet
- 1000-mL Class A volumetric flask

1. Prepare a 1.00-mg/L zinc standard solution as follows. Pipet 10.00 mL of Zinc Standard Solution, 100-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily.
2. Follow the zinc procedure.
3. To adjust the calibration curve using the reading obtained with the 1.00-mg/L standard solution, navigate to Standard Adjust in the software (**OPTIONS**>(**MORE**)>**STANDARD ADJUST**).
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Digestion

A sample digestion is required before an analysis for total zinc can be started. A digestion will make sure that all zinc compounds in the sample are in a chemical form that will be measured. Complete the following steps to digest the sample.

Note: *The following procedure is the USEPA mild digestion. See the Water Analysis Guide for more digestion procedures.*

1. If nitric acid has not been added to the sample previously, add 5 mL of concentrated nitric acid to one liter of sample (use a glass serological pipet and pipet filler). If the sample was acidified at collection, add 3 mL of nitric acid to one liter of sample.
2. Transfer 100 mL of acidified sample to a 250-mL Erlenmeyer flask.

3. Add 5 mL of 1:1 hydrochloric acid*.
4. Heat the sample on a hot plate* at 95 °C (203 °F) until 15-20 mL remain. Make sure the sample does not boil.
5. Filter the cooled sample with 0.45 µm filter to remove any insoluble material.
6. Adjust the pH of the digested sample to pH 4–5 with 5.0 N sodium hydroxide. See *Sample collection, preservation and storage* for instructions.
7. Quantitatively transfer the sample to a 100-mL volumetric flask and dilute to the mark with deionized water.

Method performance

Program	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
780	1.00 mg/L Zn	0.97–1.03 mg/L Zn	0.013 mg/L Zn

Summary of method

Zinc and other metals in the sample are complexed with cyanide. Adding cyclohexanone causes a selective release of zinc. The zinc reacts with 2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene (zincon) indicator to form a blue-colored species. The blue color is masked by the brown color from the excess indicator. The intensity of the blue color is proportional to the amount of zinc present. Test results are measured at 620 nm.

* See *Optional reagents and apparatus*.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Zinc Reagent Set, 20-mL sample size, includes:	—	—	2429300
Cyclohexanone	0.5 mL	100 mL MDB	1403332
ZincoVer® 5 Reagent Powder Pillows	1	100/pkg	2106669

Required apparatus

Description	Quantity	Unit	Catalog number
Cylinder, graduated, mixing, 25-mL	1	each	2088640
Sample cell, 10 mL, square, matched pair	2	2/pkg	2495402

Recommended standards

Description	Unit	Catalog number
Water, deionized	4 L	27256
Zinc Standard Solution, 100-mg/L	100 mL	237842
Zinc Standard Solution, 10-mg/L Voluette® Ampule, 25-mL as Zn	16/pkg	1424610
Zinc Standard Solution, 1000-mg/L	100 mL	1417742

Optional reagents and apparatus

Description	Unit	Catalog number
Flask, Erlenmeyer, 250 mL	each	50546
Hot Plate, 120 V	each	1206701
Hydrochloric Acid 6.0 N, 1:1	500 mL	88449
Nitric Acid, concentrated, ACS	500 mL	15249
Sodium Hydroxide 5.0 N	50 mL SCDB	245026
Tensette Pipet, 0.1–1.0	each	1970001
Tips for Tensette Pipet 1970001	50/pkg	2185696
Ampule Breaker	each	2196800
Pipet, volumetric, Class A, 10 mL	each	1451538
Pipet, Filter, Safety bulb	each	1465100
Flask, volumetric, Class A, 1000 mL	each	1457453
Filter paper 0.45 µm	100/pkg	1353000
pH paper test strips, 3.0–5.5 pH range	15’ roll	37333
Filtration apparatus, glass	each	234000

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Edition 7

Microbiology

Bacteria Test Guidelines

DOC316.53.01188

All tests for bacteria use a nutritional broth or agar and incubation at a specific temperature to grow the target organism. Sterile equipment and careful handling techniques are necessary to prevent contamination of the sample.

Methods for bacteria testing

The following descriptions give a general overview of the different methods for bacteria tests.

- **Presence/absence (P/A)**—the sample is added to a bottle containing broth and incubated. A color change indicates the presence of the target bacteria.
- **Most Probable Number (MPN)**—the sample is diluted and added to a series of tubes containing broth. The tubes are incubated and then examined for the presence of gas.
- **Membrane Filtration (MF)**—the sample is filtered and the filter is placed on a pad containing growth media. After incubation, the filter is examined for the growth of the target organism.
- **Plate count agar**—the sample is mixed with an agar in a large petri dish and incubated. After incubation, the agar is examined for bacteria colonies. This test is usually used for total or heterotrophic bacteria.

Presumptive and confirmation tests

Two tests are necessary for most methods, a presumptive test and a confirmation test.

- **Presumptive test**—uses growth media that facilitates the growth of the target organism. A positive result is an indication of the target organism but can include a false positive result. The P/A, MPN and MF methods are presumptive tests.
- **Confirmation test**—uses media that is more selective for the target organism and sometimes uses a higher incubation temperature. Some media, such as the m-ColiBlue24® broth used with the MF method, is selective enough for the target organism (E. coli) that no confirmation test is required.

Techniques for microbiological tests

Good laboratory technique is essential for microbiological tests. To make sure the results are reliable, collect and preserve samples carefully. Use high-quality laboratory equipment and ready-to-use media to save time and minimize errors.

Prepare the work area

- Start the incubator while preparing other materials. Set the incubator to the temperature required by the specific procedure (usually 35 ± 0.5 °C for total coliforms and enterococci and 44.5 ± 0.2 °C for fecal coliforms).
- Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
- Mark each sample container with the sample number, dilution, date and other necessary information. Avoid contaminating the inside of the sample container in any way.
- Use presterilized Whirl-Pak® bags or bottles for sample collection. If the sample has been disinfected, use bags or bottles that contain a dechlorinating agent.

Prepare sample containers

To collect samples, use any of the following: sterilized plastic bags, sterilized disposable bottles, autoclavable glass or plastic bottles.

Sterilized plastic bags or disposable bottles
Presterilized plastic bags and bottles are available with or without dechlorinating agent. The bottles are available with a 100-mL fill-to line.

Dechlorinating reagent should be used with potable or chlorinated water samples. It is not necessary for unchlorinated or non-potable water samples. However, dechlorinating reagent will not interfere with unchlorinated samples so, for simplicity, plastic bags containing dechlorinating reagent may be used for all samples.

Autoclavable glass or plastic bottles:
Glass or plastic bottles (125-mL size) may be used instead of sterilized plastic bags or disposable bottles. These containers should be prepared as follows:

1. Wash in hot water and detergent.
2. Thoroughly rinse with hot tap water, followed by a distilled water rinse to make sure that all detergent is removed.
3. If dechlorinating agent is needed (for chlorinated, potable water), add the contents of one Dechlorinating Reagent Powder Pillow for each 125-mL of container volume. (A 250-mL sample container will require two powder pillows.)
4. Steam sterilize glass and autoclavable plastic containers at 121 °C (250 °F) for 15 minutes. Glass sample containers may be sterilized by hot air at 170 °C (338° F) for one hour.
5. Store sterile containers, tightly capped, in a clean environment until needed.

Autoclave option for sterilization

Use presterilized laboratory equipment and media to save time and minimize errors. When numerous samples must be run on a routine basis, sterilization of non-disposable materials with an autoclave may be preferable.

Procedure

1. Wash sample bottles, pipets, petri dishes, filter holder with stopper and graduated cylinder (if needed) with hot water and detergent.
2. Rinse several times with tap water and then with demineralized water. Dry thoroughly.
3. Prepare all equipment for the autoclave as follows:
   - Loosely thread caps on sample bottles and cover caps and bottle necks with foil or paper.
   - Cover the openings of graduated cylinders with foil or paper.
   - Insert the filter funnel base into an autoclavable rubber stopper that will fit the filter flask.
   - Wrap the two parts of the filter funnel assembly separately in heavy wrapping paper and seal with masking tape.
   - Wrap petri dishes (borosilicate glass) in paper or place in aluminum or stainless cans.
4. Sterilize equipment in an autoclave at 121 °C (250 °F) for 15 minutes. Borosilicate glass items may be sterilized with dry heat at 170 °C (338 °F) for a minimum of 1 hour.

Collect and preserve samples

Collect a sufficient volume of sample for analysis (usually a minimum of 100 mL of sample). World Health Organization guidelines prescribe 200 mL per sample while Standard Methods for the Examination of Water and Wastewater prescribes 100 mL per sample. Avoid sample contamination.

No dechlorination is necessary if the sample is added directly to the growth medium on site. Otherwise, treat the samples to destroy chlorine residual. Sodium thiosulfate that has been sterilized within the collection vessel is used to remove chlorine residual. Transport for analysis immediately after collection.

Analyze as soon as possible after collection. Allow no more than 6 hours to elapse between collection and examination for non-potable water samples and 30 hours for potable water samples. For best results, maintain the sample at or below 10 °C, but do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

Collect at least 100 mL of sample in a presterilized plastic bag or bottle or in a sterile glass or plastic bottle. Do not fill sample containers completely. Maintain at least 2.5 cm (approximately one inch) of air space to allow adequate space for mixing the sample prior to analysis.

Faucets, spigots, hydrants or pumps
Collect representative samples by allowing the water to run from a faucet, hydrant or pump at a moderate rate for 2 to 3 minutes before sampling. Adjust the flow rate before sample collection to avoid splashing during collection. Do not adjust the rate of flow while collecting the sample. Avoid valves, spigots and faucets that swivel or leak or those with attachments such as aerators and screens or remove the attachments prior to sample collection.

Handle the sample containers carefully. Open the sample containers just prior to collection and close immediately following collection. Do not lay the lid or cap down. Do not touch the lip or inside surface of the container. Do not rinse the containers before use. Label the sample containers immediately and analyze promptly.

Rivers, lakes and reservoirs
When sampling a river, lake or reservoir, do not sample near the edge or bank. Remove the cap, grasp the sample container near the bottom and plunge the container, mouth down, into the water in order to exclude any surface scum. Fill the container entirely under water by positioning the mouth into the current or, in non-flowing water, by tilting the container slightly and allowing it to fill slowly. Do not rinse the container before use. Label the sample containers immediately and analyze promptly.

Dilute non-potable samples

Non-potable water samples must be diluted to a level at which the bacteria can be measured.

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container in a Belt to Ear motion, approximately 25 times for 30 seconds.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert the sample container in a Belt to Ear motion 25 times for 30 seconds. This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until the necessary dilution level has been reached.

Dispose of bacteria cultures

To safely dispose of bacterial cultures left in the broth tubes, use one of the following methods:

Bleach
Sterilize used test containers with household bleach. Add 1–2 mL of the bleach to each test tube. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.

Autoclave
Place used test tubes in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test tubes in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. When cool, seal the bag, place it in another garbage bag and tie tightly.

The use of indicator organisms in water tests

Many serious diseases, such as typhoid fever and dysentery, can be traced directly to pathogenic microorganisms in polluted water. These disease-producing organisms are discharged in fecal wastes and are difficult to detect in water supplies. People may come in contact with these pathogens in drinking water or in recreational waters such as swimming pools, rivers, streams and bathing beaches.

Testing directly for bacterial pathogens is impractical for many reasons, not the least of which is the need for lengthy and involved test procedures. It has become customary to use indicator organisms such as coliform bacteria instead. Indicator organisms are usually not pathogenic and are present when pathogens are present and absent when pathogens are absent. Indicator organisms are usually of fecal origin as well.

No one organism or group of organisms satisfies all of the criteria for an indicator. For example, in temperate climates total coliform bacteria are commonly used as indicator organisms in potable water supplies. In many tropical climates, however, indigenous Escherichia coli (E. coli) bacteria are present in pristine water sources where no fecal contamination exists, yet they will produce positive results in total coliform tests.

In such cases, other bacteria, known to be associated with fecal contamination, can be used as indicator organisms in place of the coliforms. Hydrogen sulfide-producing bacteria have been shown to be associated with the presence of fecal contamination and total coliform bacteria and they may be used as an indicator organism in place of coliforms.

Total coliform tests are used for potable water supplies. Fecal coliform tests are used on untreated (non-potable) water, wastewater, bathing water and swimming water.

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Edition 7

Membrane Filtration Guidelines

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The Membrane Filtration (MF) method is used to estimate bacterial populations in water that is low in turbidity. This method is especially useful for large sample volumes or for many daily tests.

Overview

The following basic steps are necessary for a membrane filtration test.

1. Non-potable water samples are diluted.
2. The sample or dilution is filtered through a membrane filter that retains the bacteria.
3. The filter is put in a petri dish on an absorbent pad that contains a nutritional broth or agar that is selective for the growth of a specific organism.
4. The petri dish containing the filter and pad is incubated for 24 hours at a specific temperature.
5. After incubation, the colonies that have grown are identified and counted.

Sample collection and sterilization

Refer to *Bacteria Test Guidelines* for instructions on sample collection and equipment sterilization.

Sample size selection

Samples that contain a high level of bacteria must be diluted so that the bacteria that grows on the filter is at a density that can be measured. Table 372 and Table 373 list recommended sample volumes for various types of samples.

Select a sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume for non-potable water or wastewater yields 20–80 coliform colonies per filter. For finished, potable water, the volume to be filtered will be 100 mL.

When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before vacuum is applied. The dilution water will help to distribute the bacteria uniformly across the membrane filter.

Table 372 Sample volume by sample type—total coliform test [1]

Sample type	100 mL	50 mL	10 mL	1 mL	0.1 mL	0.01 mL	0.001 mL	0.0001 mL
Drinking water	X							
Swimming pools	X							
Wells, springs	X	X	X					
Lakes, reservoirs	X	X	X					
Water supply intake			X	X	X			
Bathing beaches			X	X	X			
River water				X	X	X	X	
Chlorinated sewage				X	X	X		
Raw sewage					X	X	X	X

[1] *Standard Methods for the Examination of Water and Wastewater,* 19th ed., Table 9222:I, page 9–56.

Table 373 Sample volume by sample type—fecal coliform test[1]

	100 mL	50 mL	10 mL	1 mL	0.1 mL	0.01 mL	0.001 mL
Lakes, reservoirs	X	X					
Wells, springs	X	X					
Water supply intake		X	X	X			
Natural bathing waters		X	X	X			
Sewage treatment plant, secondary effluent			X	X	X		
Farm ponds, rivers				X	X	X	
Storm water run-off				X	X	X	
Raw municipal sewage					X	X	X
Feedlot run-off					X	X	X

[1] *Standard Methods for the Examination of Water and Wastewater*, 19th ed., Table 9222:III, pages 9–61.

Sample dilution

Non-potable water samples must be diluted to a level at which the bacteria can be measured. The ideal sample volume for total coliform testing yields approximately 20 to 80 coliform colonies and not more than 200 colonies of all types per filter. Ideal sample volumes for fecal coliform testing yield approximately 20 to 60 coliform colonies per filter. Analyze three different sample volumes when the coliform number is uncertain.

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container in a waist-to-ear motion, approximately 25 times (for 30 seconds).
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle. Invert the bottle in a waist-to-ear motion, approximately 25 times (for 30 seconds). This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until the necessary dilution level has been reached.

Dilution series

d. **10-mL sample:** Transfer 11 mL of sample into 99 mL of sterile Buffered Dilution Water.

e. **1-mL sample:** Transfer 11 mL of the 10-mL dilution from step d into 99 mL of sterile Buffered Dilution Water.

f. **0.1-mL sample:** Transfer 11 mL of the 1-mL dilution from step e into 99 mL of sterile Buffered Dilution Water.

g. **0.01-mL sample:** Transfer 11 mL of the 0.1-mL dilution from step f into 99 mL of sterile Buffered Dilution Water.

h. **0.001-mL sample:** Transfer 11 mL of the 0.01-mL dilution from step g into 99 mL of sterile Buffered Dilution Water.

i. **0.0001-mL sample:** Transfer 11 mL of the 0.001-mL dilution from step h into 99 mL of sterile Buffered Dilution Water.

Field filtration apparatus

The field filtration apparatus consists of disposable funnels, a portable funnel base (vacuum support) and hand pump for convenient filtration in the field. A portable incubator can be used for incubation or for transport to the laboratory.

1. Flame sterilize the top surface of the stainless steel field vacuum support.
2. Attach the syringe tip to the vacuum support tubing.
3. Using sterile forceps, place a membrane filter, grid side up, on the center of the vacuum support.

   Note: *To sterilize forceps, dip forceps in alcohol and flame in an alcohol burner. Cool before use.*
4. Remove a funnel (base first) from the package.
5. Place the funnel onto the vacuum support. Do not touch the inside of the funnel. Push evenly on the funnel's upper rim to snap it on the vacuum support.
6. Pour the sample into the funnel.
7. Use the hand pump to draw the sample through the filter apparatus.

   Note: *See specific procedures for the sample volume required.*
8. Remove the funnel.
9. Press the lever on the vacuum support stem to lift the membrane filter from the vacuum support surface.
10. Use sterile forceps to remove the membrane filter.
11. Place the membrane filter into a prepared petri dish and incubate at the specified temperature.
12. Disconnect the syringe tip from the vacuum support tubing. Dispose of the liquid in the syringe.
13. Follow step 2 through step 12 to filter remaining samples.

Accuracy check

Positive and negative controls are important. Pseudomonas aeruginosa is recommended as a negative control, and a Escherichia coli is recommended as a positive control for total and fecal coliforms. Escherichia coli is recommended as a negative control and Enterococcus faecalis is recommended as a positive control for the enterococci. Escherichia coli is recommended as a negative control and Pseudomonas aeruginosa is recommended as a positive control for pseudomonas.

Note: *Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.*

Interpreting and reporting results

Report test results as the number of colonies per 100 mL of sample.

Single filter test
Use the following equation to calculate the result from a single membrane filter. Note that "mL sample" in the equation refers to the actual sample volume and not the diluted volume.

$$\text{Bacterial colonies per 100 mL} = \frac{\text{Bacterial colonies counted}}{\text{mL of sample}} \times 100$$

- **Indistinct colonies**—If growth covers the entire filtration area of the membrane or a portion of it, and colonies are not discrete, report the test results as "Confluent growth with or without coliforms."
- **High colony density**—If the total number of colonies exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as "Too numerous to count" (TNTC).

In either case, run a new sample using a dilution that will give about 50 to 200 colonies of all types.

When testing non-potable water, if no filter meets the desired minimum colony count, use the equation under *Multiple filter test* to calculate the test result.

Multiple filter test
Use the following equation to calculate the result from multiple membrane filters such as duplicate samples or multiple dilutions of the same sample.

$$\text{Bacterial colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Prepared broth and agar

Prepared broth or agar is ready to use and is available in broth ampules or agar plates. The ampules or agar plates contain enough medium for one test. Prepared media is shipped with a Certificate of Analysis and has an expiration date printed on the label.

The ampules are available in glass or plastic. Open the ampule and pour the broth on the absorbent pad in a petri dish. Open the plastic ampules by unscrewing the top of the ampule. Open the glass ampules with an ampule breaker.

Refer to *Prepared media for membrane filtration* for a list of prepared media that is available for microbiological tests.

Table 374 Prepared media for membrane filtration

Media	Description	Cat. No.	Selectivity	Incubation	Shelf life	Approval Citations	Sample
KF-Streptococcus	Broth in plastic ampules	2812750	Enterococci	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Ground water Surface water Recreational water
m-ColiBlue24®	Prepared agar plate	2805215	Total Coliform and Escherichia coli Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	40CFR 141.21 (f) (3) footnote (11) and 40CFR 136.3 Does not apply to Agar plate	Drinking water Beverages Ground water Surface water Recreational water Wastewater
	Broth in glass ampules	2608420					
	Broth in plastic ampules	2608450					
	Broth in 100-mL glass bottle	2608442					
m-EI	Prepared agar plate	2811715	Enterococci	24 hours at 41 ± 0.5 °C	3 months at 2–8 °C	—	Drinking water Ground water Surface water Recreational water
m-Endo	Prepared agar plate	2811615	Total Coliform Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	Standard Method 18th 9222 A, B and Federal Register V 68; #139 (7/21/2003)	Drinking water Beverages Ground water Surface water Recreational water
	Broth in glass ampules	2373520					
	Broth in plastic ampules	2373550					
	Broth in 100 mL glass bottle	2373542					
m-FC	Prepared agar plate	2811515	Fecal Coliform Bacteria	24 hours at 44.5 ± 0.2 °C	1 year at 2–8 °C	40CFR 141.21 (f) (5), Standard Method 18th 9222 D and Federal Register V 68; #139 (7/21/2003)	Ground water Surface water Recreational water Wastewater
	Broth in glass ampules	2373220					
	Broth in plastic ampules	2373250					
m-FC with Rosolic Acid	Broth in glass ampules	2428520	Fecal Coliform Bacteria	24 hours at 44.5 ± 0.2 °	1 year at 2–8 °C	40CFR 141.21 (f) (6) (i) and Standard Method 18th 9221 D	Ground water Surface water Recreational water Wastewater
	Broth in plastic ampules	2428550					
m-Green YM	Broth in glass ampules	2428320	Yeast and Mold	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Beverages
	Broth in plastic ampules	2428350					
m-HPC	Prepared agar plate	2811415	Heterotrophic Bacteria	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	Standard Method 18th 9215 A, D	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	2812450					

Table 374 Prepared media for membrane filtration

Media	Description	Cat. No.	Selectivity	Incubation	Shelf life	Approval Citations	Sample
m-TEC	Agar Tubes/2 tests per tube	2561106	Escherichia coli	2 hours at 35 ± 0.5 °C then 22 hours at 44.5 ± 0.2 °C	1 year at 2–8 °C	Federal Register V 68; #139 (7/21/2003) (applies to m-TEC and Modified m-TEC)	Recreational water
m-TEC, modified	Prepared agar plate	2811815					
m-TGE	Broth in glass ampules	2373820	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	2373850					
m-TGE with TTC	Broth in glass ampules	2428420	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	2428450					
m-TSB/USP	Broth in plastic ampules	2812650	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
Nutrient Agar/MUG	Agar Tubes/2 tests per tube	2437306	Escherichia coli (confirmation)	4 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
	Prepared agar plate	2812115					
Pseudomonas	Broth in plastic ampules	2812250	Pseudomonas	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
R2A	Agar Tubes/2 tests per tube	2724106	Stressed Heterotrophic Bacteria	At least 72 hours at 35 ± 0.5 °C (7 days maximum)	1 year at 2–8 °C	Standard Method 18th 9215 A, D	Drinking water Beverages
	Prepared agar plate	2814215		At least 72 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	
	Broth in plastic ampules	2812350					

Dehydrated media

Refer to the *Dehydrated media and reagents* table for a list of dehydrated media that can be prepared. The media must be measured, mixed with water and sterilized before use.

Dehydrated media and reagents

Description	Unit	Catalog number
Brain Heart Infusion Agar	500 g	2405634
Brain Heart Infusion Broth	500 g	2815534
Magenta GlcA (5-bromo-6-chloro-3indoyl-beta-D-glucuronide)	2 g	2815035
M-E Agar	100 g	2281226
M-E Agar	500 g	2281234
m-TEC Agar, dehydrated	100 g	2281126
Nalidixic Acid	25 g	2407124
Oxidase Reagent	0.5 mL	2622500
Tryptic Soy Agar	100 g	2565926
Tryptic Soy Broth, ampules	50/pkg	2812650

Enrichment technique for total coliforms

Stressed coliforms require an enrichment technique, such as the one using Lauryl Tryptose (LT) Broth Ampules described here, to get complete recovery with the MF Method. Consult your local or state authorities about approved test methods for your application.

Procedure

1. Place a sterile absorbent pad in the lid of a sterile petri dish.
2. Saturate the pad with one LT Broth Ampule. Pour off any excess liquid.
3. Filter the sample through a membrane filter.
4. Place the membrane filter onto the saturated pad.
5. Incubate the filter without inverting the petri dish for 1.5 to 2 hours at 35 ± 0.5 °C in a relative humidity of at least 90 percent.
6. Remove the petri dish from the incubator and open it.
7. Place a sterile absorbent pad in the bottom half of the petri dish.
8. Pour the contents of one m-Endo Broth Ampule into the bottom half of the petri dish.
9. Carefully remove the filter from the lid and roll it onto the new pad to avoid trapping air between the filter and the nutrient pad.
10. Discard the old pad (the enrichment pad saturated with LT Broth). Replace the culture dish lid.
11. Invert the culture dish and incubate at 35 ±0.5 °C for 20 to 22 hours.
12. After incubation, use an illuminated magnifier or a 10 to 15X microscope to count the colonies with a greenish-gold metallic sheen.

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Edition 7

Membrane Filtration Method

Pre-poured Agar Plate

Scope and Application: water and wastewater

Test preparation

Before starting the test:

Prepared Agar Plates contain prepared media. The plates are ready to use without the need for further preparation and contain enough medium for one test.
The shelf life of the prepared agar plates varies from 3 months to 1 year (see the *Table of Specifications*). Plates are shipped with a Certificate of Analysis and have an expiration date printed on the label.
To sterilize forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

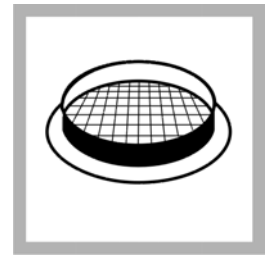
Agar plate membrane filtration

1. Set up the membrane filter apparatus.

With sterile forceps, place a membrane filter, grid side up, into the assembly.

2. Invert the sample for 30 seconds to mix. Pour 100 mL of sample into the funnel. Apply vacuum and filter the sample[1]. Rinse the funnel walls with 20 to 30 mL of sterile Buffered Dilution Water. Apply vacuum. Repeatedly rinse the funnel walls two more times.

3. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the prepared agar.



4. With a slight rolling motion, place the filter, grid side up, on the agar. Check for trapped air under the filter and make sure the filter touches the entire surface of the agar. Replace the lid on the petri dish.

Agar plate membrane filtration (continued)

5. Invert the petri dish and incubate. Refer to the *Table of Specifications* for temperature and time.

6. Remove the petri dish from the incubator and count the colonies using a 10 to 15X stereoscopic microscope.

[1] Release vacuum once dry so that the filter does not dry out and tear.

Interpreting and reporting results

Report the density as number of colonies per 100 mL of sample. Use samples that produce about 50 and not more than 200 colonies per membrane to compute density.

Use Equation A to calculate density. Note that “mL sample” refers to actual sample volume and not the volume of the dilution.

Equation A — Density on a single membrane filter.

$$\text{Colonies per 100 mL} = \frac{\text{Colonies counted}}{\text{mL sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane or a portion of it, ad colonies are not discrete, report the results as “Confluent Growth.”
- If the total number of colonies exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, a new sample must be run using a dilution that will give about 50 and not more than 200 colonies.

When testing nonpotable water, if no filter meets the desired minimum colony count, the average density can be calculated with Equation B.

Equation B — Average density for duplicates, multiple dilutions or more than one filter/ sample.

$$\text{Colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Specifications

Table 375 Table of Specifications

Prepared Agar Plate	Incubation	Sensitivity	Selectivity	Shelf Life
m-HPC	24 hours at 35 ± 0.5 °C	1 CFU/ 100 mL	Heterotrophic Bacteria	1 year, 2–8 °C
m-Endo	24 hours at 35 ± 0.5 °C	1 CFU/ 100 mL	Total Coliform Bacteria	1 year, 2–8 °C
m-FC	24 hours at 44.5 ± 0.2 °C	1 CFU/ 100 mL	Fecal Coliform Bacteria	1 year, 2–8 °C
m-ColiBlue24®	24 hours at 35 ± 0.5 °C	1 CFU/ 100 mL	Total Coliforms and *E. coli*	1 year, 2–8 °C
m-EI	24 hours at 44.5 ± 0.5 °C	1 CFU/ 100 mL	Enterococci	3 months, 2–8 °C
Modified m-TEC	2 hours at 35 ± 0.5 °C then 22 hours at 44.5 °C	1 CFU/ 100 mL	*E. coli*	1 year, 2–8 °C
Nutrient Agar/ MUG	24 hours at 35 ± 0.5 °C	1 CFU/ 100 mL	*E. coli* (confirmation)	1 year, 2–8 °C

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Agar plates, pre-poured:		
m-HPC	15/pkg	2811415
m-Endo	15/pkg	2811615
m-FC	15/pkg	2811515
m-ColiBlue24®	15/pkg	2805215
m-EI	15/pkg	2811715
m-TEC modified	15/pkg	2811815
Nutrient Agar/MUG	15/pkg	2182115

Required apparatus

Description	Unit	Catalog number
Aspirator, water	each	213102
Bags, Whirl-Pak[1] with dechlorinating agent, 180-mL	100/pkg	2075333
Counter, hand tally	each	1469600
Filter Holder, magnetic coupling	each	1352900
Filter Funnel Manifold, aluminum, 3-place	each	2486100
Filtering Flask, 1000-mL	each	54653
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Pall Gelman	200/pkg	1353001
Forceps, stainless steel	each	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 240 VAC, 50/60 Hz	each	2619202
Incubator, Water Bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 240 VAC, 50/60 Hz	each	2616302
Microscope, compound, 10X (15X available)	each	2942500
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pump, Vacuum, hand-operated	1	1428300
Stopper, rubber, one-hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ($^{5}/_{16}$ in.) ID	3.7 m (12 ft.)	55919

[1] Whirl-Pak is a registered trademark of Nasco, Inc.

Optional apparatus

Description	Unit	Catalog number
Autoclave, Automatic, 120 VAC, 50/60 Hz	each	2898600
Bottles, sample, glass, with cap, 118-mL	3/pkg	2163103
Bottles, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottles, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Graduated Cylinder, 100-mL	each	50842
Incubator, portable, 12 VDC	each	2569900
Incubator, Water Bath, with gable cover, 110 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, with gable cover, 220 VAC, 50/60 Hz	each	2616302
Magnifier, illuminated, 2.5X and 5X,	each	2585400
Magnifier, illuminated, 10X, portable	each	2585300
Marker, laboratory	each	2092000
Microscope, Compound, 10X (15X available)	each	2942500
Pad, absorbent, with dispenser	1000/pkg	1491800
Pen, colony counter (Felt-tip pen attached to a counter, that marks, beeps and registers accumulative count on an LCD display)	each	2613200

Optional apparatus (continued)

Description	Unit	Catalog number
Pump, Vacuum/Pressure, 115 VAC, 60 Hz	each	2824800
Pump, Vacuum/Pressure, 230 VAC, 50 Hz	each	2824802
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement (use with 2087760)	10/pkg	2097810
Isopropyl alcohol	500 mL	1445949
Alcohol burner	each	2087742
Battery eliminator	each	2580400
Bags, Whirl-Pak®, without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak, without dechlor 720 mL	10/pkg	1437297
Rechargeable battery pack, for portable incubator 12V DC / 115 V AC adapter	each	2580300
230V AC Rechargeable batter pack adapter (for 2580300)	each	2595902
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199

Optional media and reagents

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dilution Water, buffered, sterile, 99-mL bottles	25/pkg	1430598
Peptone Powder Pillows, 1-g	30/pkg	2142964
Potassium Dihydrogen Phosphate and Magnesium Chloride Powder Pillows for buffered dilution water (25 of each)	50/pkg	2143166
Tryptic Soy Agar	100 g	2565926

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Edition 7

Coliforms—Total, Fecal and *E. coli*

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USEPA Membrane Filtration Method[1] **Method 8074**

m-Endo

Scope and Application: For potable water, nonpotable water, recreation water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*, 9222 B and 9221 B.

Introduction

The Membrane Filtration (MF) method is a fast way to estimate bacterial populations in water. The MF method is especially useful when evaluating large sample volumes or performing many coliform tests daily.

Method

In the initial step, an appropriate sample volume passes through a membrane filter with a pore size small enough (0.45 micron) to retain the bacteria present. The filter is placed on an absorbent pad (in a petri dish) saturated with a culture medium that is selective for coliform growth. The petri dish containing the filter and pad is incubated, upside down, for 24 hours at the appropriate temperature. After incubation, the colonies that have grown are identified and counted using a low-power microscope.

PourRite™ Ampules contain prepared selective media. This eliminates the measuring, mixing, and autoclaving needed when preparing dehydrated media. The ampules are designed with a large, unrestrictive opening that allows media to pour out easily. Each ampule contains enough medium for one test.

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
If using PourRite® ampules, allow the media to warm to room temperature before opening.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Potable water must not contain any coliform bacteria. Do not dilute potable water samples.
Prepared m-Endo agar plates may be used instead of m-Endo broth.

Potable water procedures

To test potable water with the MF Method, examine a 100-mL sample for total coliforms by incubating a filter at 35 ± 0.5 °C for 22–24 hours on m-Endo Broth. Coliforms ferment lactose in the medium and produce an acid-aldehyde complex. This complex combines with Schiff's Reagent (also in the medium) to form an iridescent green coating over the colonies. When magnified 10 to 15 times, coliforms appear as dark red colonies with a greenish-gold sheen.

For replacement items, see *m-Endo Broth for presumptive total coliforms*.

Presumptive test for total coliforms (m-Endo), method 8074

1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the lid.

Do not touch the pad or the inside of the petri dish.

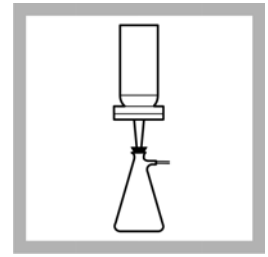
To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

For ease of use, petri dishes with pads are available.

2. Invert an m-Endo Broth PourRite Ampule 2 to 3 times to mix the broth. Use the ampule breaker to break open the ampule. Carefully pour the contents evenly over the absorbent pad. Replace the petri dish lid. Repeat steps 1 and 2 for each petri dish being prepared.

3. Set up the Membrane Filter Assembly. Use sterilized forceps to place a membrane filter, grid side up, into the assembly.

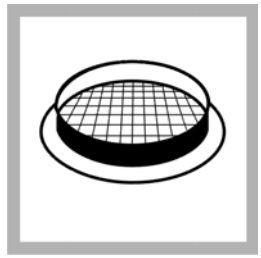
Alternatively, a sterile disposable filter unit may be used.



4. Invert the sample for 30 seconds to mix. Pour 100 mL of sample into the funnel. Apply vacuum and filter the sample. Release the vacuum. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Repeat rinsing step 2 more times.

Release the vacuum when the filter is dry to prevent damage to the filter.

5. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Check for air trapped under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Invert the petri dish and incubate at 35 ± 0.5 °C for 22–24 hours.

8. After incubating, use a 10 to 15X microscope to count the red colonies that have a greenish-gold metallic sheen.

The sheen may extend over the entire colony, or it may be localized to the edge or to the center.

Presumptive test for total coliforms (m-Endo), method 8074 (continued)

9. Record the results of the test. See *Interpreting and Reporting Results*

Depending on the test protocol, confirm positive results.

To confirm total coliforms, follow *Confirmation of total coliforms (LT and BGB), method 8074*.

To confirm fecal coliforms, follow *Confirmation of fecal coliforms (EC medium), method 8074*.

To confirm *E. coli*, follow *Confirmation of E. coli (EC or EC/MUG), method 8074.*

Confirmation of total coliforms (Lauryl Tryptose and Brilliant Green Bile)

For potable water samples, confirm typical colonies to ensure they are coliforms. (Confirm sheen colonies, up to a maximum of five.) Inoculate parallel tubes of Lauryl Tryptose (LT) single-strength (SS) Broth and Brilliant Green Bile (BGB) Broth by transferring growth from each colony. Growth and gas production in both tubes verifies that the suspect organisms are coliforms. Most Probable Number (MPN) coliform tubes are ideal for this purpose.

Use the swabbing technique for fecal coliforms or *E. coli*:

- When determining only the presence or absence of total coliforms
- When inoculating EC or EC/MUG media

Inoculate in this order:

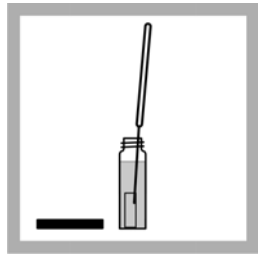
1. EC or EC/MUG
2. LT SS Broth
3. BGB

For replacement items, see *Confirmation of total coliforms (brilliant green bile broth and lauryl tryptose broth)*.

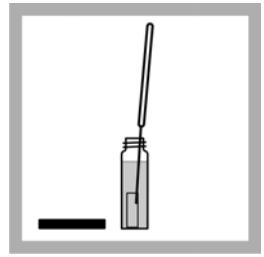
Confirmation of total coliforms (LT and BGB), method 8074

1. Sterilize an inoculating needle, or use a sterile, disposable inoculating needle.

To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner. Let the needle cool before use.



2. Touch the needle to the coliform (sheen) colony grown on m-Endo plate. Transfer to a single-strength Lauryl Tryptose (LT) Broth tube.



3. Again touch the same coliform colony with the needle. Transfer to a Brilliant Green Bile (BGB) Broth tube.

4. Invert both tubes to eliminate any air bubbles trapped in the inner vials. Incubate the tubes at 35 ± 0.5 °C. After one hour, invert the tubes to remove trapped air in the inner vial, then continue incubation.

5. After 24 ± 2 hours, check the inner vials for growth and gas bubbles. Growth (turbidity) and gas bubbles in both the LT and BGB Broth tubes verify that the colonies are coliforms. If one or both tubes do not show gas, continue incubating both tubes for an additional 24 hours

6. If no gas is present in the LT Broth tube after 48 hours, the colony is not a coliform and additional testing is unnecessary.

Record the results of the test. See *Interpreting and Reporting Results*

Confirm positive results. If growth and gas are produced in the LT Broth tube but not in the BGB Broth tube, inoculate another BGB tube from the gas-positive LT Broth tube. Incubate this BGB Broth tube and check for growth and gas after 24 hours and/or after 48 hours. If growth and gas are produced within 48 ± 3 hours, the colony is confirmed as coliform.

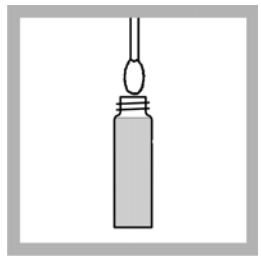
Confirmation of fecal coliforms (EC medium)

Analyze total-coliform-positive potable water samples for the presence of fecal coliform or *E. coli*. Confirm fecal coliforms from a membrane filter positive for total coliforms by swabbing the membrane with a sterile cotton swab and inoculating a tube of EC Medium Broth. Growth and gas production in the EC Medium confirms the presence of fecal coliforms.

For replacement items, see *Confirmation of fecal coliforms (EC medium broth)*.

Confirmation of fecal coliforms (EC medium), method 8074

1. Use a sterile cotton swab or inoculating loop to swab the entire surface of the total coliform-positive membrane filter (colonies grown on m-Endo Broth).



2. Swirl the cotton swab in an EC Medium Broth tube to transfer the colonies collected from the filter. Remove the cotton swab from the medium.

Use the same cotton swab to transfer colonies from the same petri dish to other broth media if desired.

3. Invert the tubes to eliminate any air bubbles trapped in the inner vial. Incubate the tube at 44.5 ± 0.2 °C. After one hour, Invert the tubes to remove trapped air in the inner vial and continue incubation.

4. After 24 ± 2 hours, check the inner vial for gas bubbles. Growth and gas bubbles in the EC Medium Broth tube confirm the presence of fecal coliforms.

5. Record the results of the test. See *Interpreting and Reporting Results*.

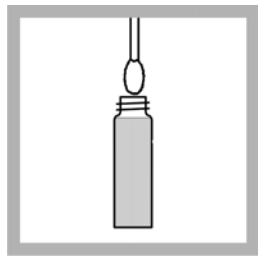
Confirmation of *E. coli* (EC or EC/MUG)

Potable water samples that test positive for total coliforms may be analyzed for the presence of *E. coli* in lieu of fecal coliforms. Use either EC medium or EC with MUG broth to confirm the presence of *E. coli* on a membrane filter positive for total coliforms.

For replacement items, see *E. coli confirmation with EC/MUG* and *E. coli confirmation with nutrient agar*.

Confirmation of *E. coli* (EC or EC/MUG), method 8074

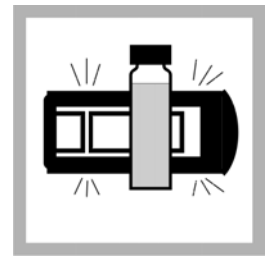
1. Use a sterile cotton swab or inoculating loop to swab the entire surface of the membrane filter that is positive for total coliforms (colonies grown on m-Endo Broth).



2. Swirl the cotton swab in an EC/MUG Broth tube to transfer the colonies collected from the filter. Remove the cotton swab from the medium.

Use the same cotton swab to transfer colonies from the same petri dish to other broth media if desired.

3. Invert the tubes to eliminate any air bubbles trapped in the inner vial. Incubate the tube at 44.5 ± 0.2 °C. After one hour, invert the tubes to remove trapped air in the inner vial and continue incubation.



4. After 24 ± 2 hours, use a long-wave UV lamp to check the tube for fluorescence. Growth and fluorescence indicate the presence of *E. coli*.

Some glass will auto-fluoresce. Use Hach brand MPN tubes for best results.

Do not look directly through the MPN tube into the UV lamp. View the tube at 90° from the lamp. Examine the tubes in a darkened area.

Have a fluorescent-positive and a fluorescent-negative tube, both with turbidity, to compare with the sample tube. Alternatively use an *E. coli* presence standard.

Confirmation of *E. coli* (EC or EC/MUG), method 8074 (continued)

5. Record the results of the test. See *Interpreting and Reporting Results*.

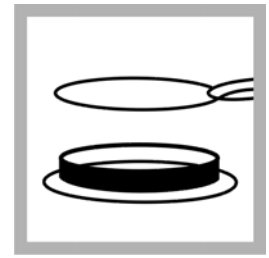
Confirmation of *E. coli* (Nutrient Agar/MUG), method 8074

1. Heat a beaker of water, or a water bath, but do not allow it to boil.

2. Loosen the cap on one or more NA/MUG nutrient agar tubes. Place the tubes into hot water. When the agar melts, carefully remove the tubes from the water with tongs.

Pre-poured agar plates can also be used.

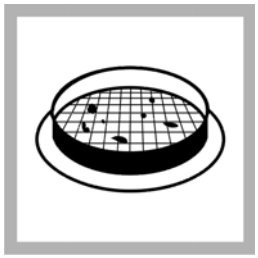
3. Using sterile technique, pour half of the contents of the tube into a sterile 47-mm petri dish. Immediately replace petri dish lid and allow agar to solidify undisturbed.



4. Use sterilized forceps to lift the membrane filter with total coliform colonies off the m-Endo absorbent pad.

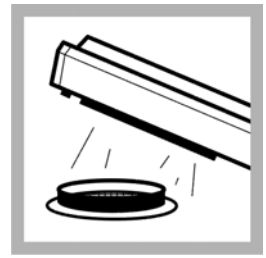
To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

Confirmation of *E. coli* (Nutrient Agar/MUG), method 8074 (continued)



5. Immediately transfer the membrane filter to the petri dish containing NA/MUG. With a slight rolling motion, center the filter, grid side up, on the agar. Check for air trapped under the filter and make sure the entire filter touches the agar. Replace the petri dish lid.

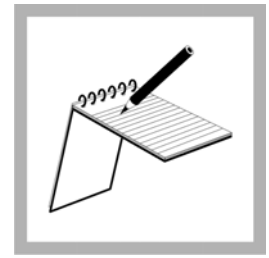
6. Invert the petri dish. Incubate for 4 hours at 35 ± 0.5 °C.



7. Using a long-wave UV lamp, examine the colonies for fluorescence. Fluorescence indicates the presence of *E. coli*.

Make sure to examine the petri dish in a darkened area.

Some UV lamps do not use the correct wattage and can give false results. Be sure to use the UV lamps and replacement bulbs that are specified in *Consumables and replacement items*.



8. Record the results of the test. See *Interpreting and Reporting Results*.

Interpreting and Reporting Results

Report coliform density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform testing, samples should produce 20 to 60 fecal coliform colonies.

Use ***Equation A*** to calculate coliform density. Note that “mL sample” refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as “Confluent Growth With or Without Coliforms.”
- If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, run a new sample using a dilution that will give about 50 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Equation B—Average coliform density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

$$\text{Coliform colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Controls:
Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Consumables and replacement items

m-Endo Broth for presumptive total coliforms

Required media and reagents

Description	Unit	Catalog number
m-Endo prepared agar plates	15/pkg	2811615
m-Endo Broth Ampules, plastic	50/pkg	2373550
m-Endo Broth, 100 mL glass bottle	1 each	2373542
m-Endo Broth PourRite™ Ampules, glass (for total coliform presumptive)	20/pkg	2373520
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	1	2087742
Ampule Breaker, PourRite™	each	2484600
Counter, hand tally	1	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	1	1352900
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	1	54653
Forceps, stainless steel	1	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Loop, inoculating, disposable	25/pkg	2749125
Microscope, compound	1	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 60 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ($^5/_{16}$ in.) ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Bags, Whirl-Pak®, with dechlorinating agent, 180 mL	100/pkg	2075333
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Incubator, portable, 12 VDC	each	2569900
Incubator, water bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, water bath, 240 VAC, 50/60 Hz	each	2616302
Isopropyl alcohol	500 mL	1445949
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

Confirmation of total coliforms (brilliant green bile broth and lauryl tryptose broth)

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

Description	Unit	Catalog number
Brilliant Green Bile Broth Tubes (for total coliform confirmation)	15/pkg	32215
Lauryl Tryptose Broth Tubes, single-strength (for total coliform confirmation)	15/pkg	2162315

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	1	2087742

Required apparatus (continued)

Description	Unit	Catalog number
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Loop, inoculating, disposable	25/pkg	2749125

Optional media, reagents and apparatus

Description	Unit	Catalog number
Ampule Breaker, PourRite™	each	2484600
Burner, Bunsen	each	2162700
Inoculating Needle, disposable	25/pkg	2748925
Lauryl Tryptose Broth Ampules, sterile (for enrichment technique)	20/pkg	1472520
Rack, coliform tube	each	221500
Wicks, replacement (used with Alcohol Burner 20877-42)	10/pkg	2097810

Confirmation of fecal coliforms (EC medium broth)

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

Description	Unit	Catalog number
EC Medium Broth Tubes (for fecal coliform confirmation)	15/pkg	1410415

Required apparatus

Description	Unit	Catalog number
Forceps, stainless steel	1	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Inoculating Needle, disposable	25/pkg	2748925
Loop, inoculating, disposable	25/pkg	2749125
Swabs, cotton, sterile (for confirmation)	100/pkg	2554300

Optional media, reagents and apparatus

Description	Unit	Catalog number
Incubator, portable, 12 VDC	1	2569900
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with North American style plug	each	2281400
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with European style plug	each	2281402
Incubator, Water Bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 240 VAC, 50/60 Hz	each	2616302
Inoculating Loop, nichrome wire, with handle	1	2112100
Rack, coliform tube	each	221500

E. coli confirmation with EC/MUG

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

Description	Unit	Catalog number
EC Medium with MUG Broth Tubes (for *E. coli* confirmation)	15/pkg	2471515

Required apparatus

Description	Unit	Catalog number
Forceps, stainless steel	1	2141100
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	1	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	1	2184302
Swabs, cotton, sterile (for confirmation)	100/pkg	2554300

Optional media, reagents and apparatus

Description	Unit	Catalog number
Ecoli Fluorescence standard	each	2361100
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	1	2569900
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Inoculating Needle, disposable	25/pkg	2748925
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with North American style plug	each	2281400
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with European style plug	each	2281402
Incubator, Water Bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 240 VAC, 50/60 Hz	each	2616302
Inoculating Loop, nichrome wire, with handle	1	2112100
Lamp, long-wave, ultraviolet, portable, 4 watt	1	2415200
Loop, inoculating, disposable	25/pkg	2749125
Rack, coliform tube	each	221500

E. coli confirmation with nutrient agar

Note: *Many of the confirmation products are also listed under the m-Endo presumptive products.*

Required media and reagents

Description	Unit	Catalog number
Nutrient Agar with MUG prepared plates	15/pkg	2182115
Nutrient Agar with MUG Tubes, 2 tests/tube (for *E. coli* confirmation)	6/pkg	2437306

Required apparatus

Description	Unit	Catalog number
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Forceps, stainless steel	1	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	1	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	1	2184302

Optional media, reagents and apparatus

Description	Unit	Catalog number
Beaker, 600 mL	1	50052
Incubator, 12-Well Dri-Bath, 115/230 VAC, 50/60 Hz with North American style plug	each	2281400
Lamp, long-wave, ultraviolet, portable, 4 watt	1	2415200

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Edition 7

Coliforms—Fecal

DOC316.53.001209

USEPA Membrane Filtration Method **Method 8074[1]**

m-FC and m-FC/RA

Scope and Application: For potable water, nonpotable water, recreation water and wastewater.

[1] USEPA approved 9222 D.

Introduction

The Membrane Filtration (MF) method is a fast way to estimate bacterial populations in water. The MF method is especially useful when evaluating large sample volumes or performing many coliform tests daily.

Method

In the initial step, an appropriate sample volume passes through a membrane filter with a pore size small enough (0.45 micron) to retain the bacteria present. The filter is placed on an agar plate prepared with a culture medium that is selective for coliform growth. The petri dish is incubated, upside down, for 24 hours at the appropriate temperature. After incubation, the colonies that have grown are identified and counted using a low-power microscope.

PourRite™ Ampules contain prepared selective media. This eliminates the measuring, mixing, and autoclaving needed when preparing dehydrated media. The ampules are designed with a large, unrestrictive opening that allows media to pour out easily. Each ampule contains enough medium for one test.

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
If using PourRite™ ampules, allow the media to warm to room temperature before opening.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

Nonpotable waters procedures

Wastewater, river, bathing, and other nonpotable waters usually are tested for fecal coliforms. In testing for fecal coliforms, a special medium and an elevated incubation temperature inhibit growth of nonfecal coliforms. Fecal coliforms growing on the membrane form an acid that reacts with an aniline dye in the medium, producing a blue color.

Use m-FC Broth with Rosolic Acid to increase specificity when high levels of non-coliform bacteria may be present, unless all the organisms in the sample are stressed or injured.

Confirmation of fecal coliforms (m-FC or m-FC/RA), method 8074

1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the petri dish lid.

Do not touch the pad or the inside of the petri dish.

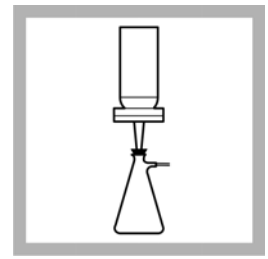
To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

Petri dishes with pads are available.

2. Invert an m-FC Broth PourRite Ampule 2 to 3 times to mix the broth. Use the ampule breaker to open an ampule. Carefully pour the contents evenly onto the absorbent pad. Replace the petri dish lid.

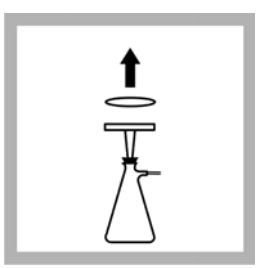
Use m-FC Broth with Rosolic Acid to increase specificity when high levels of non-coliform bacteria may be present, unless the organisms are stressed or injured.

3. Set up the Membrane Filter Assembly. Use sterilized forceps to place a membrane filter, grid side up, into the assembly.

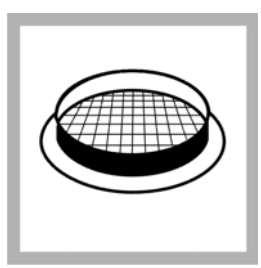


4. Prepare the necessary dilutions to obtain the proper sample size. Invert the sample for 30 seconds to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Repeat rinsing step, two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.



5. Turn off the vacuum and lift off the funnel top. Use sterile forceps to transfer the membrane filter to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Check for air trapped under the filter and make sure the entire filter touches the pad. Replace the petri dish lid.

7. Invert the petri dish and incubate at 44.5 ± 0.2 °C for 24 ± 2 hours.

To eliminate environmental Klebsiella from the fecal coliform population elevate the temperature to 45.0 ± 0.2 °C.

Alternatively, a water bath with rack may be used for incubation by placing the petri dishes into a sealed bag.

8. After incubating, count the blue colonies using a 10 to 15X microscope.

Confirmation of fecal coliforms (m-FC or m-FC/RA), method 8074 (continued)

9. Record the results of the test. See *Interpreting and reporting results*.

To verify results, follow *Verifying fecal coliforms, method 8074*

Confirmation of total coliforms (Lauryl Tryptose and Brilliant Green Bile)

For potable water samples, confirm typical colonies to ensure they are coliforms. (Confirm sheen colonies, up to a maximum of five.) Inoculate parallel tubes of Lauryl Tryptose (LT) Single Strength (SS) Broth and Brilliant Green Bile (BGB) Broth by transferring growth from each colony. Growth and gas production in both tubes verifies that the suspect organisms are coliforms. Most Probable Number (MPN) coliform tubes are ideal for this purpose.

Use the swabbing technique for fecal coliforms or *E. coli*:

- When determining only the presence or absence of total coliforms
- When inoculating EC or EC/MUG media

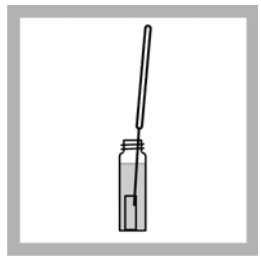
Inoculate in this order:

1. EC or EC/MUG
2. LT SS Broth
3. BGB

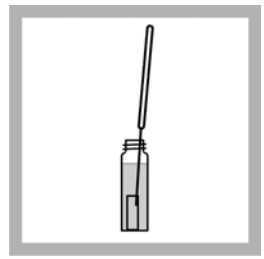
Confirmation of total coliforms (LT and BGB), method 8074

1. Sterilize an inoculating needle, or use a sterile, disposable inoculating needle.

To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner. Let the needle cool before use.

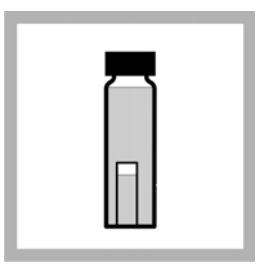


2. Touch the needle to the coliform (sheen) colony grown on m-Endo Broth. Transfer to a single-strength Lauryl Tryptose (LT) Broth tube.



3. Again touch the same coliform colony with the needle. Transfer to a Brilliant Green Bile (BGB) Broth tube.

4. Invert both tubes to eliminate any air bubbles trapped in the inner vials. Incubate the tubes at 35 ± 0.5 °C. After one hour, invert the tubes to remove trapped air in the inner vial, then continue incubation.



5. After 24 ± 2 hours, check the inner vials for growth and gas bubbles. Growth (turbidity) and gas bubbles in both the LT and BGB Broth tubes verify that the colonies are coliforms. If one or both tubes do not show gas, continue incubating both tubes for an additional 24 hours

6. If no gas is present in the LT Broth tube after 48 hours, the colony is not a coliform and additional testing is unnecessary.

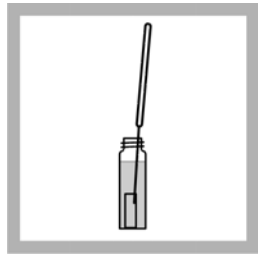
Record the results of the test. See *Interpreting and reporting results*

Confirm positive results. If growth and gas are produced in the LT Broth tube but not in the BGB Broth tube, inoculate another BGB tube from the gas-positive LT Broth tube. Incubate this BGB Broth tube and check for growth and gas after 24 hours and/or after 48 hours. If growth and gas are produced within 48 ± 3 hours, the colony is confirmed as coliform.

Verifying fecal coliforms, method 8074

1. Sterilize an inoculating needle, or use a sterile, disposable inoculating needle.

To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner flame. Let the needle cool before use.



2. Touch the needle to a typical blue colony and transfer to a Lauryl Tryptose (LT) Broth tube. Repeat steps 1 and 3 for each test being verified.

Steps 3 and 4 can be performed simultaneously if multiple incubators are available.

3. Invert the tubes to eliminate air trapped inside the inner vials. Incubate the tubes at 35 ± 0.5 °C. After one hour, invert the tubes to remove trapped air in the inner vial and continue incubation. Check tubes for growth and gas production at 24 hours. If no change has occurred, continue incubation for another 24 hours.

If growth and gas are not produced in 48 ± 3 hours, the colony was not coliform. If growth and gas are produced in 48 ± 3 hours, use a sterile loop to inoculate one EC Medium Broth tube from each gas-positive LT Broth tube.

4. Invert the tubes to eliminate air trapped inside the inner vials. Incubate the EC Medium tubes at 44.5 ± 0.2 °C for 24 ±2 hours. After one hour, invert the tubes to remove trapped air in the inner vial.

5. Growth and gas production at 44.5 °C within 24 ± 2 hours confirms the presence of fecal coliforms.

Record the results of the test. See *Interpreting and reporting results*.

Interpreting and reporting results

Report coliform density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform testing, samples should produce 20 to 60 fecal coliform colonies.

Use ***Equation A*** to calculate coliform density. Note that “mL sample” refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as “Confluent Growth With or Without Coliforms.”
- If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, run a new sample using a dilution that will give about 50 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Equation B—Average coliform density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

$$\text{Coliform colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Controls:
Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Consumables and replacement items

Confirmation of fecal coliforms (m-FC or m-FC/RA)

Required media and reagents

Description	Unit	Catalog number
m-FC prepared agar plates	15/pkg	2811515
m-FC Broth Ampules, plastic	50/pkg	2373250
m-FC w/Rosolic Acid Broth Ampules, plastic	50/pkg	2428550
m-FC Broth PourRite™ Ampules (for fecal coliform presumptive)	20/pkg	2373220
m-FC with Rosolic Acid Broth PourRite™ Ampules (fecal coliform presumptive)	20/pkg	2428520

Required apparatus

Description	Unit	Catalog number
Ampule Breaker, PourRite™	each	2484600
Counter, hand tally	1	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	1	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	1	2486100
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	1	54653
Forceps, stainless steel	1	2141100
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Inoculating Needle, disposable	25/pkg	2748925
Loop, inoculating, disposable	25/pkg	2749125
Microscope, compound	each	2942500

Optional media and reagents

Description	Unit	Catalog number
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Incubator, Water Bath, 110 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 220 VAC, 50/60 Hz	each	2616302

Confirmation of total coliforms (brilliant green bile broth and lauryl tryptose broth)

Required media and reagents

Description	Unit	Catalog number
Brilliant Green Bile Broth Tubes (for total coliform confirmation)	15/pkg	32215
Lauryl Tryptose Broth Ampules, sterile (for enrichment technique)	20/pkg	1472520
Lauryl Tryptose Broth Tubes, single-strength (for total coliform confirmation)	15/pkg	2162315

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	1	2087742
Ampule Breaker, PourRite™	each	2484600
Burner, Bunsen	each	2162700
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Isopropyl alcohol	500 mL	1445949
Loop, inoculating, disposable	25/pkg	2749125
Pad, absorbent, with dispenser	1000/pkg	1491800

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Bags, Whirl-Pak®, with dechlorinating agent, 180 mL	100/pkg	2075333
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ID	3.7 m (12 ft)	56019
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	10/pkg	2097810

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Edition 7

Coliforms—*E. coli*

DOC316.53.001210

USEPA Membrane Filtration Method **Method 8367[1]**

m-TEC

Scope and Application: For potable water, nonpotable water, recreation water and wastewater.

[1] USEPA accepted.

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.

The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.

Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

m-TEC Agar confirmation for presumptive *E. coli* test

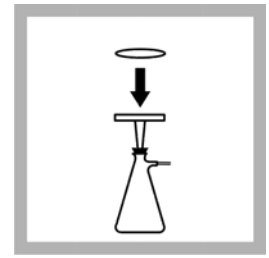
The m-TEC method detects E. coli in recreational fresh water samples with a two step process. First, membrane filters are incubated on m-TEC Agar for 2 hours at 35 °C to resuscitate injured organisms. The thermotolerant organisms are then selected by fermentation of lactose at an elevated temperature of 44.5 °C. The second step uses a substrate medium containing urea to distinguish urease-negative E. coli from other thermotolerant coliforms that hydrolyze urea. Yellow or yellow-brown urease-negative colonies are positive for E. coli.

Presumptive *E. coli* test (m-TEC Agar), method 8367

1. Heat a beaker of water or water bath but do not allow it to boil.

2. Place m-TEC Agar tubes into hot water. When agar melts, carefully remove tubes from hot water with tongs.

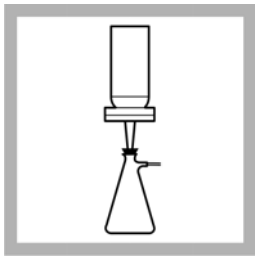
3. Using sterile technique, pour half of the contents of the tube into a sterile, 47-mm petri dish. Immediately replace the petri dish lid and allow agar to solidify undisturbed.



4. Set up the Membrane Filter Assembly. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

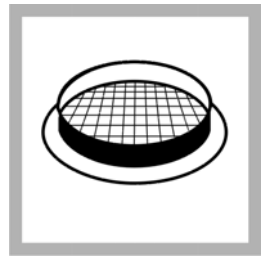
Presumptive *E. coli* test (m-TEC Agar), method 8367



5. Prepare the necessary dilutions to obtain the proper sample size. Invert the sample for 30 seconds to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Repeat the rinsing step, two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.

6. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the previously prepared petri dish.



7. With a slight rolling motion, place the filter, grid side up, on the agar. Check for air trapped under the filter and make sure the entire filter touches the agar. Replace the petri dish lid.

8. Invert the petri dish. Incubate at 35 ± 0.5 °C for 2 hours and then at 44.5 ± 0.2 °C for 22 hours.

Presumptive *E. coli* test (m-TEC Agar), method 8367

9. To confirm, use sterilized forceps to transfer the filter to a pad saturated with at least 2 mL of urea substrate.

Preparing the urea substrate:

a. Dissolve 2.0 g of urea in 100 mL of deionized water.

b. Add 10 mg of phenol red sodium indicator to the urea solution.

c. Use 0.02 N sulfuric acid to adjust the pH to between 3 and 4. The solution will turn yellow.

d. Store solution at 2 to 8 °C. Use within one week.

10. After 15 minutes, count yellow or yellow-brown colonies by using a 10 to 15X microscope. The presence of yellow or yellow-brown colonies confirms *E. coli*.

11. Record the results of the test. See *Interpreting and reporting results*.

Interpreting and reporting results

Report coliform density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform testing, samples should produce 20 to 60 fecal coliform colonies.

Use ***Equation A*** to calculate coliform density. Note that “mL sample” refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as “Confluent Growth With or Without Coliforms.”
- If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, run a new sample using a dilution that will give about 50 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Equation B—Average coliform density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

$$\text{Coliform colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Controls:
Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-TEC Agar Tubes, 2 tests/tube (for *E. coli* determination)	6/pkg	2561106
Phenol Red Sodium Salt	5 g	2563922
Sulfuric Acid, 0.02 N	100 mL	20342
Urea Reagent, ACS	100 g	1123726

Required apparatus

Description	Unit	Catalog number
Counter, hand tally	each	1469600
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	each	1352900
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Microscope, Compound	each	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Isopropyl alcohol	500 mL	1445949
m-ColiBlue24® Broth, 100 mL glass bottle	1 each	2608442
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	—	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Coliforms—*E. coli*

DOC316.53.001211

USEPA Membrane Filtration Method **Method 8367[1]**

modified m-TEC

Scope and Application: For potable water, nonpotable water, recreation water and wastewater

[1] USEPA accepted

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.

The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.

Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

Modified m-TEC prepared Agar Plates for *E. coli* in recreational waters

A revised single-step method uses modified m-TEC Agar to detect *E. coli* in recreational fresh water samples. The modified m-TEC Agar contains an enzymatic substrate which is cleaved to produce colonies. Red or magenta colored colonies specifically represent the presence of *E. coli*. Confirmation steps are not required with this method.

Modified m-TEC for *E. coli* in recreational waters, method 8367

1. Set up the Membrane Filter Assembly. Refer to *Introduction to Coliforms*. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

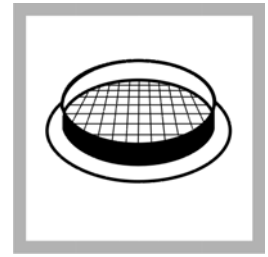
2. Prepare the necessary dilutions to obtain 20–80 E. coli colonies on the membranes.

Invert the sample for 30 seconds to mix.

Pour the sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Repeat rinsing step, two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.

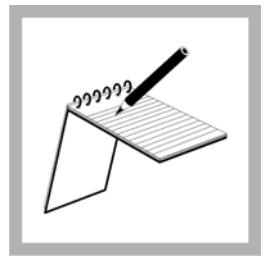
3. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the modified m-TEC prepared agar plate.



4. With a slight rolling motion, place the filter, grid side up, on the agar plate. Check for air trapped under the filter and make sure the entire filter touches the agar. Replace the petri dish lid.

5. Invert the petri dish. Incubate at 35 ±0.5 °C for 2 hours, then at 44.5 ±0.2 °C for 22 hours.

6. Count the number of red or magenta colonies by using a 10 to 15X microscope.



7. The presence of these colonies confirms *E. coli*. Record the results of the test. See *Interpreting and reporting results*.

Interpreting and reporting results

Report coliform density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform testing, samples should produce 20 to 60 fecal coliform colonies.

Use ***Equation A*** to calculate coliform density. Note that “mL sample” refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as “Confluent Growth With or Without Coliforms.”
- If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, run a new sample using a dilution that will give about 50 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Equation B—Average coliform density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

$$\text{Coliform colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Controls:
Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-TEC, modified, prepared agar plates, 47-mm	15/pkg	2811815

Required apparatus

Description	Unit	Catalog number
Counter, hand tally	each	1469600
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	each	1352900
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Microscope, Compound	each	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Incubator, water bath, 120 VAC, 50/60 Hz	each	2616300
Isopropyl alcohol	500 mL	1445949
m-ColiBlue24® Broth, 100 mL glass bottle	1 each	2608442
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	—	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Coliforms—Total and *E. coli*

DOC316.53.001213

USEPA Membrane Filtration Method **Method 10029[1]**

m-ColiBlue24®

Scope and Application: For potable water, nonpotable water, recreation water and wastewater.

[1] USEPA approved.

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
If using PourRite™ ampules, allow the media to warm to room temperature before opening.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

m-ColiBlue24 Broth PourRite Ampules

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages; surface, well, and groundwater, waste water, recreational waters and process water for ultrapure, chemical processing and pharmaceutical applications.

Simultaneous total coliform and *E. coli* screening, method 10029

1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish.

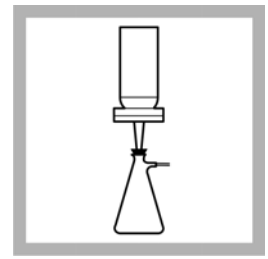
Do not touch the pad or the inside of the petri dish.

To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

2. Invert ampules two or three times to mix broth. Break open an ampule of m-ColiBlue24 Broth using an ampule breaker. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

3. Set up the Membrane Filter Apparatus. With sterile forceps, place a membrane filter, grid side up, into the assembly.

Alternatively, a sterile disposable filter unit may be used.

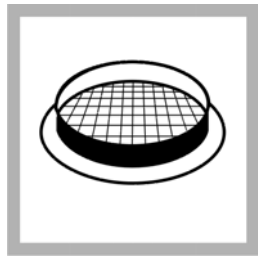


4. Invert the sample for 30 seconds to mix. Pour 100 mL of sample or diluted sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Rinse again two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.

Simultaneous total coliform and *E. coli* screening, method 10029 (continued)

5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Invert the petri dish and incubate at 35 ± 0.5 °C for 24 hours.

8. Remove the petri dish from the incubator and examine the filters for colony growth. Colonies are typically readily visible; however, a microscope or other 10–15X magnifier may be useful. Red and blue colonies indicate total coliforms and blue colonies specifically indicate *E. coli*.

Sometimes only the center of a colony will show color. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as a blue colony. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as *E. coli.*

Optional testing of red colonies

The m-ColiBlue24 Broth is formulated so that coliforms other than *E. coli* grow as red colonies. The percentage of red colonies that are false positives (non-coliforms) is comparable to the percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms); therefore, confirmation is not required.

A few varieties of the non-coliform bacteria *Pseudomonas, Vibrio,* and *Aeromonas* spp. may grow on m-ColiBlue24 Broth and form red colonies. Such bacteria can be readily distinguished from total coliforms by the oxidase test. *Pseudomonas, Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If your sample contains high levels of interfering bacteria, you can perform an oxidase test to confirm which red colonies are total coliforms.

Two oxidase procedures are provided. Count the red and blue colonies on the m-ColiBlue24 Broth membrane filter before starting the oxidase test.

Oxidase method 1
This method enables you to conveniently and rapidly evaluate membrane filters that have numerous colonies. Use this method after 24 hours of incubation on m-ColiBlue24 Broth.

Research* shows that the oxidase test cannot be performed on media that undergoes acidification during bacterial growth. The m-ColiBlue24 Broth is formulated so that the medium does not undergo such acidification. Consequently, many colonies can be simultaneously tested for their oxidase reaction using the following procedure.

1. Remove the lid from the petri dish containing the m-ColiBlue24 Broth membrane filter, invert the lid, and place it on the bench top.

   Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive controls and *Escherichia coli* for negative controls. Use Aqua QC-Stiks™ for quality control procedures.

2. Drop approximately 0.5 mL of Difco SpotTest™ Oxidase Reagent into the center of the inverted lid.

3. Using sterile forceps, transfer the membrane filter from the pad and place the filter upright in the inverted lid.

4. Within 10 to 15 seconds, the oxidase reagent will soak into the filter and cause the oxidase-positive colonies to turn purple. This purple color may be visible in the colony itself or adjacent to the colony. Oxidase-negative colonies will retain the red color they developed when incubated on m-ColiBlue24 Broth.

5. After the initial 10 to 15 second reaction time, start counting the red colonies that turn purple. Count individual colonies by using a microscope with 10–15X magnification

   Note: *To simplify colony counting place a spare lid on the lid containing the oxidase reagent and membrane filter. Use a felt-tip pen to mark the lid as you identify the purple colonies. After 30 seconds, you can count marks that indicate purple (oxidase-positive) colonies.*

6. Stop counting 30 seconds after initial 10 to 15 second reaction time, because oxidase-negative colonies will start to develop a purple color.

   Note: *Bacteria are not killed with this procedure, so colonies may be selected for streaking and for additional testing.*

Colonies that are blue after the initial 24-hour incubation on m-ColiBlue24 Broth are almost always *E. coli* and do not need confirmation with the oxidase procedure.

Oxidase method 2
This method is the official oxidase test described in *Standard Methods for the Examination of Water and Wastewater,* 18th edition, 1992.

1. Select red colonies from an m-ColiBlue24 Broth membrane filter and streak onto Tryptic Soy Agar.

2. Incubate Tryptic Soy Agar plates at 35 °C (95 °F) for 18–24 hours or until isolated colonies are obtained.

* A.H. Havelaar et al. 1980. False-negative oxidase reaction as a result of medium acidification. *Antonie van Leeuwenhoek*. 46, 301-312. L.K. Hunt et al. 1981. Role of pH in oxidase variability of *Aeromonas hydrophila. Journal of Clinical Microbiology*. 13: 1054-1059.

Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive and *Escherichia coli* for negative controls. Use Aqua QC-Stiks™* for quality control procedures.

3. Saturate a piece of filter paper with Difco SpotTest Oxidase Reagent. (This reagent contains a stabilized solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride.)

   Note: *Alternatively, oxidase reagent can be dropped directly onto colonies growing on Tryptic Soy Agar. Oxidase-positive colonies will turn from pink to purple.*

4. Using a sterile nichrome inoculating needle, transfer cellular material from an isolated Tryptic Soy Agar colony to the moist filter paper.

   Note: *Do not use iron or other reactive needles for inoculation, because they will cause false-positive results. Wooden applicator sticks work well.*

5. Oxidase-negative colonies will not react with the reagent, but oxidase-positive colonies will cause the reagent to turn dark purple within 10 seconds.

6. Oxidase-negative colonies should be counted as total coliform bacteria.

Interpreting and reporting results

Report coliform density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform testing, samples should produce 20 to 60 fecal coliform colonies.

Use ***Equation A*** to calculate coliform density. Note that "mL sample" refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as "Confluent Growth With or Without Coliforms."
- If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as "Too Numerous To Count" (TNTC).

In either case, run a new sample using a dilution that will give about 50 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Equation B—Average coliform density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

$$\text{Coliform colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Controls:
Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

* Aqua QC-Stiks is a trademark of MicroBiologics.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-ColiBlue24® Broth Ampules, glass	20/pkg	2608420
m-ColiBlue24® Broth Ampules, plastic	50/pkg	2608450
m-ColiBlue24® prepared agar plates	15/pkg	2805215

Required apparatus

Description	Unit	Catalog number
Ampule Breaker, PourRite™	each	2484600
Bags, Whirl-Pak®, with dechlorinating agent, 180 mL	100/pkg	2075333
Counter, hand tally	each	1469600
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	each	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	each	2486100
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Microscope, Compound	each	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ($^{5}/_{16}$ in.) ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Incubator, water bath, 120 VAC, 50/60 Hz	each	2616300
Isopropyl alcohol	500 mL	1445949
m-ColiBlue24® Broth, 100 mL glass bottle	1 each	2608442
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	—	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Enterococci

DOC316.53.001212

Membrane Filtration Method **Proposed Method 1600**

M-EI

Scope and Application: For potable water, nonpotable water, recreation water and wastewater.

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for bacteria testing yields 20–80 colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

m-EI prepared Agar Plates for Enterococci test in recreational waters

Enterococci are used as an indicator organism for recreational water quality evaluation. The m-EI Agar specifically detects enterococci in 24 hours after enzymatic cleavage of a substrate (indoxyl-β-D-glucoside) which results in formation of colonies surrounded by a blue halo.

m-EI for Enterococci, proposed method 1600

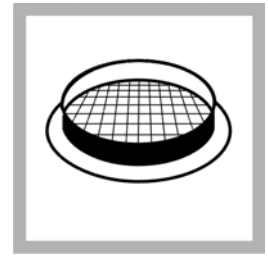
1. Set up the Membrane Filter Assembly. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

2. Prepare the necessary dilutions to obtain the proper sample size. Invert the sample for 30 seconds to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Rinse again two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.

3. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the previously prepared agar plate.

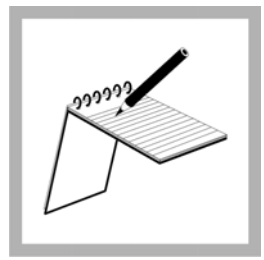


4. With a slight rolling motion, place the filter, grid side up, on the prepared m-EI agar plate. Check for air trapped under the filter and make sure the entire filter touches the agar.

Replace the petri dish lid.

5. Invert the petri dish. Incubate at 41 ± 0.5 °C for 24 hours.

6. Count any colonies (regardless of color) with a blue halo. Use a 10 to 15X microscope.



7. Record these colonies as enterococci. See *Interpreting and reporting results*.

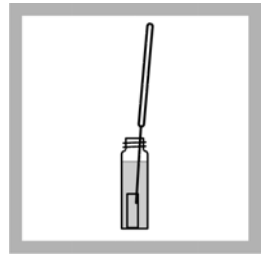
Optional verification of Enterococci

Several media can be used to verify the presence of enterococci grown on m-EI Agar. Gram-positive cocci, such as enterococci, will grow and hydrolyze esculin on Bile Esculin Agar, which results in the formation of a black or brown precipitate. Enterococci can also be confirmed by their ability to grow in Brain Heart Infusion Broth at an elevated incubation temperature of 45 °C and in Brain Heart Infusion Broth containing 6.5% NaCl.

Brain and heart infusion broth for optional detection

1. Use a sterile inoculating needle to transfer cells from the centers of at least 10 well-isolated typical colonies into a Brain Heart Infusion Broth (BHIB) tube and onto a Brain Heart Infusion Agar (BHIA) slant.

2. Incubate broth tubes for 24 hours and agar slants for 48 hours at 35 ± 0.5 °C.



3. After 24 hours, transfer a loopful of broth from the BHIB tube to each of the following media and incubate for 48 hours:

Bile Esculin Agar (BEA) and incubate at 35 ± 0.5 °C.

BHIB and incubate at 45 ± 0.5 °C.

BHIB with 6.5% NaCl and incubate at 35 ± 0.5 °C.

4. After 48 hours observe all media for growth and apply a Gram stain to the growth from each BHIA slant.

5. Gram-positive cocci that grow and hydrolyze esculin on BEA (i.e. produce a black or brown precipitate) and grow in BHIB and BHIB with salt are verified as Enterococci.

Record the results of the test. See *Interpreting and reporting results*

Interpreting and reporting results

Report enterococci density as the number of colonies per 100 mL of sample. Use samples that produce 20 to 80 enterococci colonies and not more than 200 colonies of all types per membrane.

Use ***Equation A*** to calculate enterococci density. Note that “mL sample” refers to actual sample volume and not volume of the dilution.

Equation A—Enterococci density on a single membrane filter

$$\text{Colonies per 100 mL} = \frac{\text{Colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane or a portion of it and colonies are not discrete, report results as “Confluent Growth With or Without Enterococci.”
- If the total number of colonies exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, run a new sample using a dilution that will give about 50 enterococci colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average enterococci density with Equation B.

Equation B—Average enterococci density for 1) duplicates, 2) multiple dilutions or 3) more than one filter/sample

$$\text{Colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-EI prepared agar plates, 47-mm	15/pkg	2811715

Required apparatus

Description	Unit	Catalog number
Counter, hand tally	1	1469600
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	1	1352900
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	1	54653
Forceps, stainless steel	1	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Loop, inoculating, disposable	25/pkg	2749125
Microscope, Compound	each	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bile Esculin Agar	500 g	2815634
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Brain Heart Infusion Agar	500 g	2405634
Brain Heart Infusion Broth	500 g	2815534
Bunsen burner with tubing	each	2162700
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Inoculating Needle, disposable	25/pkg	2748925
Incubator, portable, 12 VDC	each	2569900
Incubator, 12-well Dri-Bath, 120 VAC 50/60 Hz	each	2281400
Isopropyl alcohol	500 mL	1445949
m-ColiBlue24® Broth, 100 mL glass bottle	1 each	2608442
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	—	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Heterotrophic Bacteria

DOC316.53.01195

Membrane Filtration Method **Method 8242**

m-TGE Broth with TTC Indicator

Scope and Application: For water and wastewater

Test preparation

Introduction

The Membrane Filter (MF) Heterotrophic Plate Count Method is a way to estimate bacterial populations in water. Since no single medium can satisfy the growth requirements of all bacteria, several types of media are offered for detecting heterotophic bacteria in water. The m-HPC medium, available in both the broth and agar formats, is a high-nutrient medium used to enumerate heterotrophs in treated potable water samples. The m-TGE broth, originally developed for use with dairy products, is now commonly used to determine bacterial counts in water by membrane filtration. The m-TGE broth with TTC contains a redox dye, triphenyltetrazolium chloride, which colors the colonies red, thus enhancing their visibility. The m-TSB/USP broth is a general purpose medium which was designed to conform with the formula specified in the USEPA's Code of Federal Regulations (21 CFR) for sterility testing of pharmaceutical products.

Convenient packaging
Hach's m-TGE and m-TGE Broth with TTC come prepared and packaged in glass or plastic ampules. Prepared ampules eliminate measuring, mixing and autoclaving steps necessary for preparing dehydrated medium. Break off the top of the ampule and pour the medium onto an absorbent pad in a petri dish.

Each ampule contains enough selective medium for one test. Packaged medium, when stored under prescribed conditions, has a shelf-life of one year. Ampules are shipped with a Certificate of Analysis and have an expiration date printed on the label.

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
If using PourRite® ampules, all the media should be warm to room temperature before opening.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

m-TGE broth with TTC indicator

1. Place a sterile, absorbent pad in a sterile petri dish (use sterile forceps to do this) and replace the lid or use a sterile petri dish with pad.

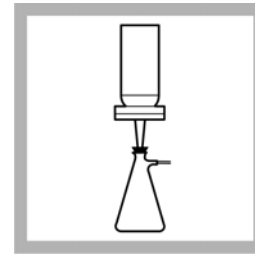
Be careful not to touch the pad or the inside of the petri dish.

For ease of use, petri dishes with pads are available.

2. Open an ampule of m-TGE with TTC Indicator and carefully pour the contents evenly over the absorbent pad.

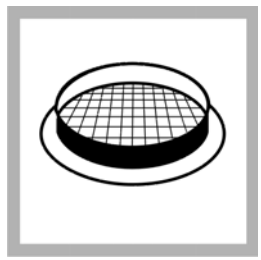
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter in the assembly, making sure the grid side is up.

Alternatively, a sterile, disposable filter unit may be used.



4. Mix the water sample by inverting for 30 seconds. Filter the appropriate volume through the sterile 47-mm, 0.45-µm, gridded membrane filter, under partial vacuum[1]. Rinse the funnel, using 20 to 30 mL portions of sterile buffered dilution water. Apply vacuum. Rinse the funnel again for two more times.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Be sure air is not trapped under the filter and that the filter touches the pad at all points. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ±0.5 °C for 24 hours.

8. Remove the dish from the incubator. Count colonies on membrane filters using a 10 to 15X microscope.

Colonies grown with m-TGE with TTC Indicator appear pink to red aid visibility.

[1] Release the vacuum once dry so that the filter does not dry out or tear.

Interpreting and reporting results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time and the medium. For example: 98 CFU/mL, membrane filter method, 35 °C, 24 hours, m-TGE Broth.

Follow these counting and recording guidelines, based on the number of colonies (on the average) per square:

1 to 2 or fewer colonies per square:

Count all of the colonies on the filter and divide the results by the volume of original sample used. Example: if there are 122 colonies on the filter and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{122 \text{ colonies}}{10 \text{ mL sample}} = 12.2 \text{ CFU/mL}$$

3 to 10 colonies per square—Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if you calculated an average of 8 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{8 \text{ colonies/square x } 100}{0.1 \text{ mL sample}} = 8000 \text{ CFU/mL}$$

10 to 20 colonies per square—Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if there are an average of 17 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows: $\frac{17 \text{ colonies/square} \times 100}{0.1 \text{ mL sample}} = 17{,}000 \text{ CFU/mL}$

More than 20 colonies per square—If there are more than 20 colonies per square, record the count as >2000 divided by the volume of original sample used.

> Example—If the original sample volume was 0.01 mL, results would be >2000/0.01 or >200000 CFU/mL.

Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.

Summary of method

In the initial step, an appropriate sample volume is passed through a membrane filter with a pore size small enough (0.45 microns) to retain the bacteria present. The filter is placed either on an absorbent pad (in a petri dish) saturated with a culture medium or on an agar medium that is selective for heterotrophic bacteria growth. The petri dish containing the filter and pad is incubated, upside down, for 24 to 48 hours, depending on the medium used, at the appropriate temperature. After incubation, the colonies which have developed are identified and counted by using a low-power microscope. The MF method is especially useful for testing drinking water because large volumes of sample can be analyzed in a short time.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-TGE Broth with TTC PourRite Ampules	20/pkg	2428420
Dilution Water, Buffered, sterile, 99 mL[1]	25/pkg	1430598

[1] Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	each	2087742
Ampule Breaker, PourRite	each	2484600
Aspirator	each	213102
Bags, Whirl-Pak with dechlorinating reagent, sterile, 180-mL	100/pkg	2075333
Counter, hand tally	each	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Filter Holder, magnetic coupling (use with 2486100)	each	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Germicidal Cloths	50/pkg	2463200
Graduated Cylinder, 100-mL	each	50842
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Incubator, Water Bath, 110 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 220 VAC, 50/60 Hz	each	2616302
Membrane Filters, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Microscope Compound	each	2942500
Pump, Vacuum, hand-operated	each	1428300
Stopper, Rubber, one hole, No. 8	6/pkg	211908
Tubing, Rubber, 0.8 cm (5/16") ID, 3.7 m (12 ft.)	each	56019
Petri dish with pad, 47-mm, Millipore	150/pkg	2936300
Millipore membrane Filters, 0.45-m, gridded, sterile	150/pkg	2936100
Isopropyl alcohol	500 mL	1445949
Pad, absorbent, with dispenser	1000/pkg	1491800
Pump, Vacuum/Pressure, Portable, 115 V 60 Hz	each	2824800
Pump, Vacuum/Pressure, Portable, 220 V 50 Hz	each	2824802

Optional media and reagents

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Potassium Dihydrogen Phosphate and Magnesium Chloride Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Sodium Thiosulfate, ACS grade	454 g	46001

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

Optional media and reagents

Description	Unit	Catalog number
Aspirator, water	each	213102
Replacement wicks for 2087742	each	2097810
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon	100/pkg	2811199
Bags, Whirl-Pak®, without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak, without dechlor 720 mL	10/pkg	1437297
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bottles, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottles, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Bunsen burner with tubing	each	2162700
Incubator, portable, 12 VDC	each	2569900
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Petri dish, 47 mm, sterile, disposable	500/pkg	1485200
Petri dish, 47 mm, sterile, disposable	100/pkg	1485299
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Rechargeable battery pack, for portable incubator 12 V DC / 115 V AC adapter	each	2580300
230 V AC Rechargeable batter pack adapter (for 2580300)	each	2595902
Battery eliminator	each	2580400

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Edition 7

Heterotrophic Bacteria

DOC316.53.01194

Membrane Filtration Method **Method 8242**

m-TGE Broth

Scope and Application: For water and wastewater

Test preparation

Introduction

The Membrane Filter (MF) Heterotrophic Plate Count Method is a fast, simple way to estimate bacterial populations in water. Since no single medium can satisfy the growth requirements of all bacteria, several types of media are offered for detecting heterotophic bacteria in water. The m-HPC medium, available in both the broth and agar formats, is a high-nutrient medium used to enumerate heterotrophs in treated potable water samples. The m-TGE broth, originally developed for use with dairy products, is now commonly used to determine bacterial counts in water by membrane filtration. The m-TGE broth with TTC contains a redox dye, triphenyltetrazolium chloride, which colors the colonies red, thus enhancing their visibility. The m-TSB/USP broth is a general purpose medium which was designed to conform with the formula specified in the USEPA's Code of Federal Regulations (21 CFR) for sterility testing of pharmaceutical products.

Convenient packaging
Hach's m-TGE and m-TGE Broth with TTC come prepared and packaged in glass or plastic ampules. Prepared ampules eliminate measuring, mixing and autoclaving steps necessary for preparing dehydrated medium. Simply break off the top of the ampule and pour the medium onto an absorbent pad in a petri dish.

Each ampule contains enough selective medium for one test. Packaged medium, when stored under prescribed conditions, has a shelf-life of one year. Ampules are shipped with a Certificate of Analysis and have an expiration date printed on the label.

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
If using PourRite® ampules, all the media should be warm to room temperature before opening.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

m-TGE broth

1. Place a sterile, absorbent pad in a sterile petri dish (use sterile forceps to do this) and replace the lid or use a sterile petri dish with pad.

Be careful not to touch the pad or the inside of the petri dish.

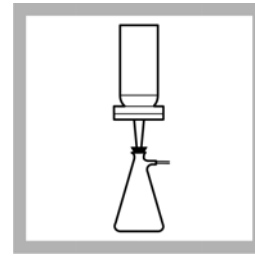
For ease of use, petri dishes with pads are available.

Alternatively, a prepoured m-HPC agar plate may be used.

2. Open an ampule of m-HPC and carefully pour the contents evenly over the absorbent pad.

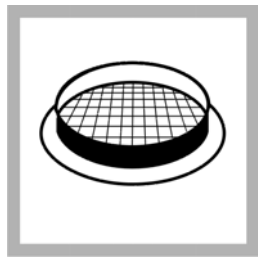
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter in the assembly, making sure the grid side is up.

Alternatively, a sterile, disposable filter unit may be used.



4. Mix the water sample by inverting for 30 seconds. Filter the appropriate volume through the sterile 47-mm, 0.45-µm, gridded membrane filter, under partial vacuum[1]. Rinse the funnel, using 20 to 30 mL portions of sterile buffered dilution water. Apply vacuum. Rinse the funnel again for two more times.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Be sure air is not trapped under the filter and that the filter touches the pad at all points. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ±0.5 °C for 48 hours.

8. Remove the dish from the incubator. Count the clear to cream colored colonies on membrane filters using a 10 to 15X microscope.

[1] Release the vacuum once dry so that the filter does not dry out or tear.

Interpreting and reporting results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time and the medium. For example: 98 CFU/mL, membrane filter method, 35 °C, 24 hours, m-TGE Broth.

Follow these counting and recording guidelines, based on the number of colonies (on the average) per square:

1 to 2 or fewer colonies per square:

Count all of the colonies on the filter and divide the results by the volume of original sample used. Example: if there are 122 colonies on the filter and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{\text{122 colonies}}{\text{10 mL sample}} = \text{12.2 CFU/mL}$$

3 to 10 colonies per square—Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if you calculated an average of 8 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{8 colonies/square x 100}}{\text{0.1 mL sample}} = \text{8000 CFU/mL}$$

10 to 20 colonies per square—Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if there are an average of 17 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows: $\frac{\text{17 colonies/square} \times 100}{\text{0.1 mL sample}} = \text{17,000 CFU/mL}$

More than 20 colonies per square—If there are more than 20 colonies per square, record the count as >2000 divided by the volume of original sample used.

Example—If the original sample volume was 0.01 mL, results would be >2000/0.01 or >200000 CFU/mL.

Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.

Summary of method

In the initial step, an appropriate sample volume is passed through a membrane filter with a pore size small enough (0.45 microns) to retain the bacteria present. The filter is placed either on an absorbent pad (in a petri dish) saturated with a culture medium or on an agar medium that is selective for heterotrophic bacteria growth. The petri dish containing the filter and pad is incubated, upside down, for 24 to 48 hours, depending on the medium used, at the appropriate temperature. After incubation, the colonies which have developed are identified and counted by using a low-power microscope. The MF method is especially useful for testing drinking water because large volumes of sample can be analyzed in a short time.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-TGE Broth PourRite Ampules	50/pkg	2373850
Dilution Water, Buffered, sterile, 99 mL[1]	25/pkg	1430598

[1] Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	each	2087742
Ampule Breaker, PourRite	each	2484600
Bags, Whirl-Pak with dechlorinating reagent, sterile, 180-mL	100/pkg	2075333
Counter, hand tally	each	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Filter Holder, magnetic coupling (use with 2486100)	each	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Germicidal Cloths	50/pkg	2463200
Graduated Cylinder, 100-mL	each	50842
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Incubator, Water Bath, 110 VAC, 50/60 Hz	each	2616200
Incubator, Water Bath, 220 VAC, 50/60 Hz	each	2616202
Gelman Membrane Filters, 0.45-µm, gridded, sterile	200/pkg	1353001
Microscope, Compound	each	2942500
Pipets, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pump, Vacuum, hand-operated	each	1428300
Stopper, Rubber, one hole, No. 8	6/pkg	211908
Tubing, Rubber, 0.8 cm (5/16") ID, 3.7 m (12 ft.)	each	56019
Petri dish with pad, 47-mm, Millipore	150/pkg	2936300
Millipore membrane Filters, 0.45-m, gridded, sterile	150/pkg	2936100
Isopropyl alcohol	500 mL	1445949
Pad, absorbent, with dispenser	1000/pkg	1491800
Pump, Vacuum/Pressure, Portable, 115 V 60 Hz	each	2824800
Pump, Vacuum/Pressure, Portable, 220 V 50 Hz	each	2824802

Optional media and reagents

Description	Unit	Catalog number
Aspirator, water	each	213102
Replacement wicks for 2087742	each	2097810
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Potassium Dihydrogen Phosphate and Magnesium Chloride Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon	100/pkg	2811199
Bags, Whirl-Pak®, without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak, without dechlor 720 mL	10/pkg	1437297
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bottles, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottles, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Incubator, portable, 12 VDC	each	2569900
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Petri dish, 47 mm, sterile, disposable	500/pkg	1485200
Petri dish, 47 mm, sterile, disposable	100/pkg	1485299
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Rechargeable battery pack, for portable incubator 12 V DC / 115 V AC adapter	each	2580300
230 V AC Rechargeable batter pack adapter (for 2580300)	each	2595902
Battery eliminator	each	2580400

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water. Autoclave (sterilize) to prepare the American Public Health Association buffered dilution water.

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Edition 7

Heterotrophic Bacteria

DOC316.53.01192

Membrane Filtration Method **Method 8242**

m-HPC, m-TSP-USP, m-TGE Broth or m-TGE Broth with TTC Indicator **m-HPC**

Scope and Application: For water and wastewater

Test preparation

Introduction

The Membrane Filter (MF) Heterotrophic Plate Count Method* is a fast, simple way to estimate bacterial populations in water. Since no single medium can satisfy the growth requirements of all bacteria, several types of media are offered for detecting heterotophic bacteria in water. The m-HPC medium, available in both the broth and agar formats, is a high-nutrient medium used to enumerate heterotrophs in treated potable water samples. The m-TGE broth originally developed for use with dairy products, is now commonly used to determine bacterial counts in water by membrane filtration. The m-TGE broth with TTC contains a redox dye, triphenyltetrazolium chloride, which colors the colonies red, thus enhancing their visibility. The m-TSB/USP broth is a general purpose medium which was designed to conform with the formula specified in the USEPA's Code of Federal Regulations (21 CFR) for sterility testing of pharmaceutical products.

Convenient packaging

Hach's m-TGE and m-TGE Broth with TTC come prepared and packaged in glass or plastic ampules. Prepared ampules eliminate measuring, mixing and autoclaving steps necessary for preparing dehydrated medium. Simply break off the top of the ampule and pour the medium onto an absorbent pad in a petri dish.

Each ampule contains enough selective medium for one test. Packaged medium, when stored under prescribed conditions, has a shelf-life of one year. Ampules are shipped with a Certificate of Analysis and have an expiration date printed on the label.

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution.
Wash hands thoroughly with soap and water.

* Method 8242

m-HPC media

1. Place a sterile, absorbent pad in a sterile petri dish (use sterile forceps to do this) and replace the lid or use a sterile petri dish with pad.

Be careful not to touch the pad or the inside of the petri dish.

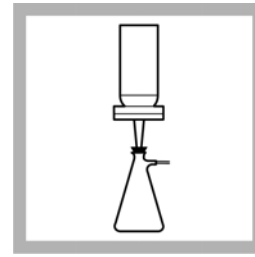
For ease of use, petri dishes with pads are available.

Alternatively, a prepoured m-HPC agar plate may be used.

2. Open an ampule of m-HPC and carefully pour the contents evenly over the absorbent pad.

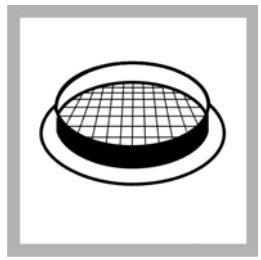
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter in the assembly, making sure the grid side is up.

Alternatively, a sterile, disposable filter unit may be used.



4. Mix the water sample by inverting for 30 seconds. Filter the appropriate volume through the sterile 47-mm, 0.45-µm, gridded membrane filter, under partial vacuum[1]. Rinse the funnel, using 20 to 30 mL portions of sterile buffered dilution water. Apply vacuum. Repeat rinsing two more times.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Be sure air is not trapped under the filter and that the filter touches the pad at all points. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ±0.5 °C for 48 hours.

8. Remove the dish from the incubator. Count the clear to cream colored colonies on the membrane filters using a 10 to 15X microscope.

[1] Release vacuum once dry so that the filter does not dry out and tear.

Interpreting and reporting results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time and the medium. For example: 98 CFU/mL, membrane filter method, 35 °C, 24 hours, m-TGE Broth.

Follow these counting and recording guidelines, based on the number of colonies (on the average) per square:

1 to 2 or fewer colonies per square:

Count all of the colonies on the filter and divide the results by the volume of original sample used. Example: if there are 122 colonies on the filter and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{122 \text{ colonies}}{10 \text{ mL sample}} = 12.2 \text{ CFU/mL}$$

3 to 10 colonies per square—Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if you calculated an average of 8 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{8 \text{ colonies/square x } 100}{0.1 \text{ mL sample}} = 8000 \text{ CFU/mL}$$

10 to 20 colonies per square—Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if there are an average of 17 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows: $\frac{17 \text{ colonies/square} \times 100}{0.1 \text{ mL sample}} = 17{,}000 \text{ CFU/mL}$

More than 20 colonies per square—If there are more than 20 colonies per square, record the count as >2000 divided by the volume of original sample used.

> Example—If the original sample volume was 0.01 mL, results would be >2000/0.01 or >200000 CFU/mL.

Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.

Summary of method

> In the initial step, an appropriate sample volume is passed through a membrane filter with a pore size small enough (0.45 microns) to retain the bacteria present. The filter is placed either on an absorbent pad (in a petri dish) saturated with a culture medium or on an agar medium that is selective for heterotrophic bacteria growth. The petri dish containing the filter and pad is incubated, upside down, for 24 to 48 hours, depending on the medium used, at the appropriate temperature. After incubation, the colonies which have developed are identified and counted by using a low-power microscope. The MF method is especially useful for testing drinking water because large volumes of sample can be analyzed in a short time.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Dilution Water, Buffered, sterile, 99 mL[1]	25/pkg	1430598
m-HPC Agar Plates	15/pkg	2811415
m-HPC Broth Ampules, plastic, 2-mL	50/pkg	2812450

[1] Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	each	2087742
Bags, Whirl-Pak with dechlorinating reagent, sterile, 180-mL	100/pkg	2075333
Counter, hand tally	each	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Filter Holder, magnetic coupling (use with 2486100)	each	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Germicidal Cloths	50/pkg	2463200
Graduated Cylinder, 100-mL	each	50842
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Incubator, Water Bath, 110 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 220 VAC, 50/60 Hz	each	2616302
Gelman, Membrane Filters, 0.45-µm, gridded, sterile	200/pkg	1353001
Microscope, Compound	each	2942500
Pipets, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pump, Vacuum, hand-operated	each	1428300
Stopper, Rubber, one hole, No. 8	6/pkg	211908
Tubing, Rubber, 0.8 cm (5/16") ID, 3.7 m (12 ft.)	each	56019
Petri dish with pad, 47-mm, Millipore	150/pkg	2936300
Millipore membrane Filters, 0.45-m, gridded, sterile	150/pkg	2936100
Pump, Vacuum/Pressure, Portable, 115v, 60 Hz	each	2824800
Pump, Vacuum/Pressure, Portable, 220v, 50 Hz	each	2824802

Optional media and reagents

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Potassium Dihydrogen Phosphate and Magnesium Chloride Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Sodium Thiosulfate, ACS grade	454 g	46001
Aspirator, water 1445949 - Isopropyl alcohol, 500 mL	each	213102
Sterilization Indicator, Sterikon®,	15/pkg	2811115
Sterilization Indicator, Sterikon	100/pkg	2811199
Bags, Whirl-Pak®, without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak, without dechlor 720 mL	10/pkg	1437297
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bottles, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottles, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Incubator, portable, 12 VDC	each	2569900
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Petri dish, 47 mm, sterile, disposable	500/pkg	1485200
Petri dish, 47 mm, sterile, disposable	100/pkg	1485299
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Rechargeable battery pack, for portable incubator 12V DC / 115 V AC adapter	each	2580300
230V AC Rechargeable batter pack adapter (for 2580300)	each	2595902
Battery eliminator	each	2580400
Pad, absorbent, with dispenser	1000/pkg	1491800
Replacement wicks for 2087742	each	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Heterotrophic Bacteria

DOC316.53.01193

Membrane Filtration Method **Method 8242**

m-TSB-USP

Scope and Application: For water and wastewater

Test preparation

Introduction

The Membrane Filter (MF) Heterotrophic Plate Count Method is a fast, simple way to estimate bacterial populations in water. Since no single medium can satisfy the growth requirements of all bacteria, several types of media are offered for detecting heterotophic bacteria in water. The m-HPC medium, available in both the broth and agar formats, is a high-nutrient medium used to enumerate heterotrophs in treated potable water samples. The m-TGE broth, originally developed for use with dairy products, is now commonly used to determine bacterial counts in water by membrane filtration. The m-TGE broth with TTC contains a redox dye, triphenyltetrazolium chloride, which colors the colonies red, thus enhancing their visibility. The m-TSB/USP broth is a general purpose medium which was designed to conform with the formula specified in the USEPA's Code of Federal Regulations (21 CFR) for sterility testing of pharmaceutical products.

Convenient packaging
Hach's m-TGE and m-TGE Broth with TTC come prepared and packaged in glass or plastic ampules. Prepared ampules eliminate measuring, mixing and autoclaving steps necessary for preparing dehydrated medium. Break off the top of the ampule and pour the medium on an absorbent pad in a petri dish.

Each ampule contains enough selective medium for one test. Packaged medium, when stored under prescribed conditions, has a shelf-life of one year. Ampules are shipped with a Certificate of Analysis and have an expiration date printed on the label.

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. .
Wash hands thoroughly with soap and water.

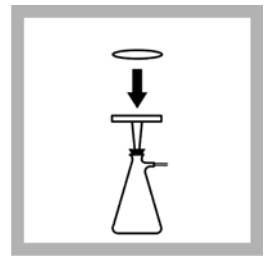
m-TSB-USP media

1. Place a sterile, absorbent pad in a sterile petri dish (use sterile forceps to do this) and replace the lid or use a sterile petri dish with pad.

Be careful not to touch the pad or the inside of the petri dish.

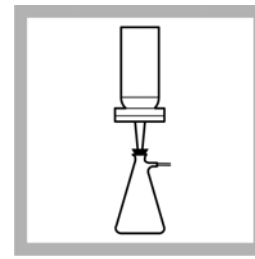
For ease of use, petri dishes with pads are available.

2. Open an ampule of m-TSB-USP and carefully pour the contents evenly over the absorbent pad.



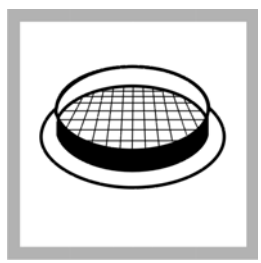
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter in the assembly, making sure the grid side is up.

Alternatively, a sterile, disposable filter unit may be used.



4. Mix the water sample by inverting for 30 seconds. Filter the appropriate volume through the sterile 47-mm, 0.45-µm, gridded membrane filter, under partial vacuum[1]. Rinse the funnel, using 20 to 30 mL portions of sterile buffered dilution water. Apply vacuum. Rinse the funnel again for two more times.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Be sure air is not trapped under the filter and that the filter touches the pad at all points. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ±0.5 °C for 24 hours.

8. Remove the dish from the incubator. Count the clear to cream colored colonies on membrane filters using a 10 to 15X microscope.

[1] Release vacuum once dry so that the filter does not dry out and tear

Interpreting and reporting results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time and the medium. For example: 98 CFU/mL, membrane filter method, 35 °C, 24 hours, m-TGE Broth.

Follow these counting and recording guidelines, based on the number of colonies (on the average) per square:

1 to 2 or fewer colonies per square:

Count all of the colonies on the filter and divide the results by the volume of original sample used. Example: if there are 122 colonies on the filter and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{\text{122 colonies}}{\text{10 mL sample}} = \text{12.2 CFU/mL}$$

3 to 10 colonies per square—Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if you calculated an average of 8 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{8 colonies/square x 100}}{\text{0.1 mL sample}} = \text{8000 CFU/mL}$$

10 to 20 colonies per square—Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used. Example: if there are an average of 17 colonies per square and the volume of original sample used was 0.1 mL, compute results as follows: $\frac{\text{17 colonies/square} \times 100}{\text{0.1 mL sample}} = \text{17,000 CFU/mL}$

More than 20 colonies per square—If there are more than 20 colonies per square, record the count as >2000 divided by the volume of original sample used.

Example—If the original sample volume was 0.01 mL, results would be >2000/0.01 or >200000 CFU/mL.

Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.

Summary of method

In the initial step, an appropriate sample volume is passed through a membrane filter with a pore size small enough (0.45 microns) to retain the bacteria present. The filter is placed either on an absorbent pad (in a petri dish) saturated with a culture medium or on an agar medium that is selective for heterotrophic bacteria growth. The petri dish containing the filter and pad is incubated, upside down, for 24 to 48 hours, depending on the medium used, at the appropriate temperature. After incubation, the colonies which have developed are identified and counted by using a low-power microscope. The MF method is especially useful for testing drinking water because large volumes of sample can be analyzed in a short time.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-TSB/USP Broth Ampules, plastic, 2-mL	50/pkg	2812650
Dilution Water, Buffered, sterile, 99 mL[1]	25/pkg	1430598

[1] Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	each	2087742
Bags, Whirl-Pak with dechlorinating reagent, sterile, 180-mL	100/pkg	2075333
Counter, hand tally	each	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Filter Holder, magnetic coupling (use with 2486100)	each	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Germicidal Cloths	50/pkg	2463200
Graduated Cylinder, 100-mL	each	50842
Incubator, Culture, low profile, 110 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220 VAC, 50/60 Hz	each	2619202
Incubator, Water Bath, 110 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 220 VAC, 50/60 Hz	each	2616302
Gelman Membrane Filters, 0.45-µm, gridded, sterile	200/pkg	1353001
Microscope Compound	each	2942500
Pipets, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pump, Vacuum, hand-operated	each	1428300
Stopper, Rubber, one hole, No. 8	6/pkg	211908
Tubing, Rubber, 0.8 cm (5/16") ID, 3.7 m (12 ft.)	each	56019
Petri dish with pad, 47-mm, Millipore	150/pkg	2936300
Millipore membrane Filters, 0.45-m, gridded, sterile	150/pkg	2936100
Isopropyl alcohol	500 mL	1445949
Pad, absorbent, with dispenser	1000/pkg	1491800
Pump, Vacuum/Pressure, Portable, 115 V 60 Hz	each	2824800
Pump, Vacuum/Pressure, Portable, 220 V 50 Hz	each	2824802

Optional media and reagents

Description	Unit	Catalog number
Aspirator, water	each	213102
Replacement wicks for 2087742	each	2097810
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Potassium Dihydrogen Phosphate and Magnesium Chloride Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon	100/pkg	2811199
Bags, Whirl-Pak®, without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak, without dechlor 720 mL	10/pkg	1437297
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bottles, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottles, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Incubator, portable, 12 VDC	each	2569900
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Petri dish, 47 mm, sterile, disposable	500/pkg	1485200
Petri dish, 47 mm, sterile, disposable	100/pkg	1485299
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Rechargeable battery pack, for portable incubator 12 V DC / 115 V AC adapter	each	2580300
230 V AC Rechargeable batter pack adapter (for 2580300)	each	2595902
Battery eliminator	each	2580400

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water. Autoclave (sterilize) to prepare the American Public Health Association buffered dilution water.

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Edition 7

Pseudomonas

DOC316.53.001214

Membrane Filtration Method **Methods 8026**

Pseudomonas Broth

Scope and Application: For potable water, nonpotable water, recreation water and wastewater

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for bacteria testing yields 20–80 colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

Pseudomonas Broth for detection of Pseudomonas species

Some species of pseudomonads are opportunistic pathogens that can inhabit recreational waters such as swimming pools and hot tubs. Pseudomonas Broth is formulated to isolate species of pseudomonas. Pseudomonas Broth contains components that promote the growth of the pigment pyocyanin, which differentiates *Pseudomonas aeruginosa* from other species by forming a blue or blue-green color. Other species of pseudomonas grow on this medium without the colony color formation.

Pseudomonas broth, method 8026

1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the petri dish lid.

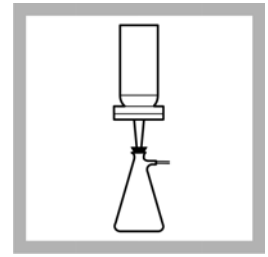
Do not touch the pad or the inside of the petri dish.

To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

2. Invert an ampule containing Pseudomonas Broth 2 to 3 times to mix. Twist the cap to break it open. Carefully pour the contents evenly onto the absorbent pad. Replace the petri dish lid. Repeat steps 1 and 2 for each petri dish.

3. Set up the Membrane Filter Assembly. Use sterilized forceps to place a membrane filter, grid side up, into the assembly.

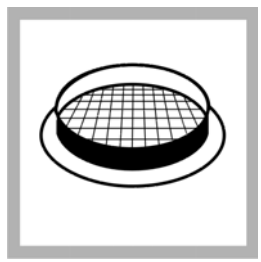
Alternatively, a sterile disposable filter unit may be used.



4. Invert the sample for 30 seconds to mix. Pour 100 mL of sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Rinse again two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.

5. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Check for air trapped under the filter and make sure that the filter touches the entire pad. Replace the petri dish lid.

7. Invert the petri dish and incubate at 35 ± 0.5 °C for 22–24 hours.

8. After incubating, count the colonies using an illuminated magnifier or a 10 to 15X microscope.

Pseudomonas broth, method 8026 (continued)

9. Colonies present on the filter after incubation are pseudomonas species. Colonies with a blue-green, green or yellow-green color are *Pseudomonas aeruginosa*.

Record the results of the test. See *Interpreting and reporting results*.

Interpreting and reporting results

Report pseudomonas density as the number of colonies per 100 mL of sample. Use samples that produce 20 to 80 pseudomonas colonies and not more than 200 colonies of all types, per membrane to compute pseudomonas density.

Use ***Equation A*** to calculate pseudomonas density. Note that “mL sample” refers to actual sample volume and not volume of the dilution.

Equation A—Pseudomonas density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane or a portion of it and colonies are not discrete, report results as “Confluent Growth With or Without Pseudomonas.”
- If the total number of colonies exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as “Too Numerous To Count” (TNTC).

In either case, run a new sample using a dilution that will give about 50 pseudomonas colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average pseudomonas density with Equation B.

Equation B—Average pseudomonas density for 1) duplicates, 2) multiple dilutions or 3) more than one filter/sample

$$\text{Colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Pseudomonas Broth Ampules, plastic	50/pkg	2812250
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598

Required apparatus

Description	Unit	Catalog number
Counter, hand tally	1	1469600
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman®	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	each	1352900
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman®	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Microscope, compound	each	2942500
Pump, hand vacuum	each	1428300
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Bags, Whirl-Pak®, with dechlorinating agent, 180 mL	100/pkg	2075333
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Isopropyl alcohol	500 mL	1445949
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	—	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Non-potable water samples must be diluted so that a MPN test will have three consecutive dilutions that contain both positive and negative tubes.

If all of the tubes in a MPN test are positive, the sample must be diluted several more times and the test must be repeated. If all of the tubes in a MPN test are negative, the sample was diluted too many times. Repeat the test with less serial dilutions.

Sterile buffered dilution water

Use dilution water that is buffered to a neutral pH and sterilized for microbiological testing. Hach dilution water is recommended for dilution of most non-potable and wastewater samples. This solution is packaged in 99 mL bottles. Each bottle contains 99 mL of sterile buffered dilution water. When 11 mL of sample is added to a 99-mL bottle of dilution water, the sample is diluted by a factor of 10 (10-fold or 10x dilution). Be sure to mix the bottles thoroughly before and after the sample is added. The dilution factor of an undiluted sample is 1.

Serial sample dilutions

Complete the following procedure to make serial dilutions of the sample. Refer to *Serial dilutions for non-potable water, MPN test* for an illustrated dilution procedure.

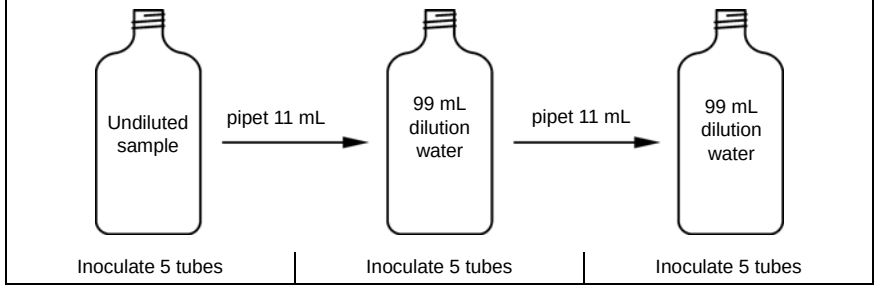
Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container vigorously for 30 seconds, approximately 25 times, using a waist-to-ear range of motion.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert (for 30 seconds) 25 times. This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until there are three bottles that contain the dilutions listed under *Serial dilutions for non-potable water, MPN test*.

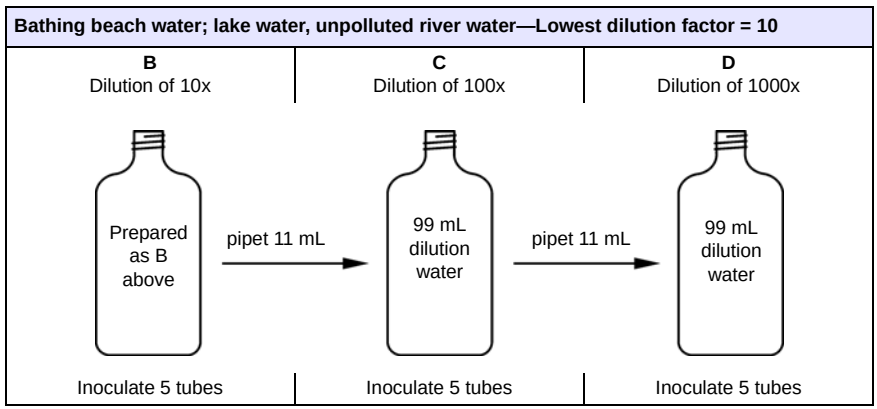
Note: *Shaking the sample too vigorously will injure or stress the organisms.*

Serial dilutions for non-potable water, MPN test

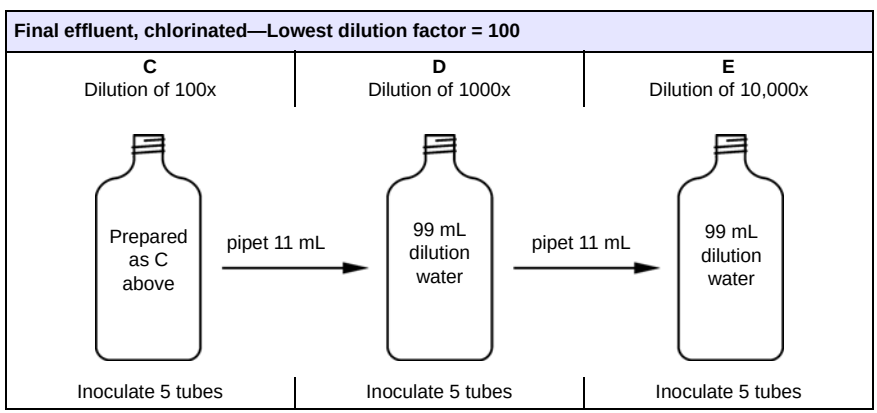
Swimming pool water, chlorinated—Lowest dilution factor = 1



Bathing beach water; lake water, unpolluted river water—Lowest dilution factor = 10

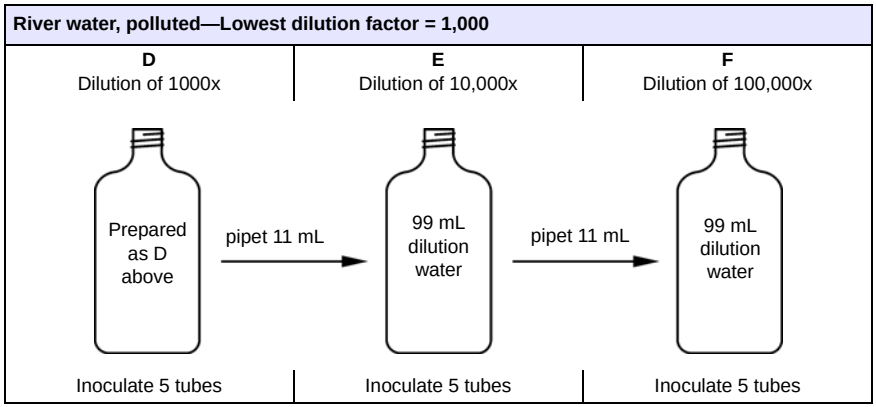


Final effluent, chlorinated—Lowest dilution factor = 100

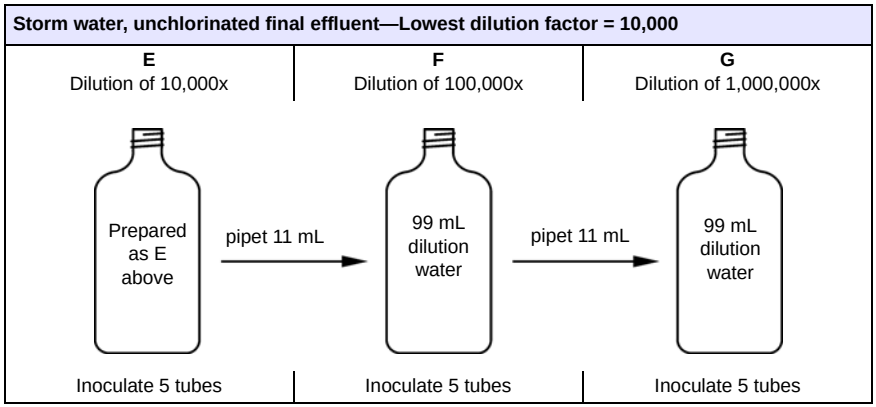


Serial dilutions for non-potable water, MPN test (continued)

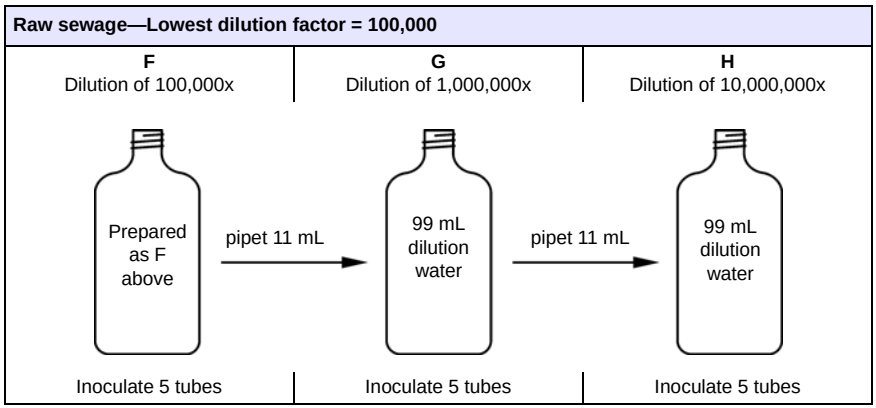
River water, polluted—Lowest dilution factor = 1,000



Storm water, unchlorinated final effluent—Lowest dilution factor = 10,000



Raw sewage—Lowest dilution factor = 100,000



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Edition 7

Coliforms—Fecal

DOC316.53.01215

USEPA[1] A-1 Medium

Method 8368

Most Probable Number (MPN) Method

Scope and Application: For non-potable water and wastewater.

[1] Most Probable Number Method 8368, A-1 Medium, is USEPA-accepted for testing non-potable waters. Method 8368 meets or exceeds the specification criteria stated in *Standard Methods for the Examination of Water and Wastewater*, 18th edition, 9221 E. Fecal Coliform Procedure. *USEPA Manual for the Certification of Laboratories Analyzing Drinking Water* states "5.5.3. A-1 medium may be used as an alternative to EC Medium to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Rule. A-1 Medium must not be used for drinking water samples."

Before starting the test:

Make sure that all materials used for containing or transferring samples are sterile.
The bottles of dilution water contain 99 mL of sterile buffered dilution water. When 11 mL of sample is added to a 99-mL bottle of dilution water, the sample is diluted by a factor of 10 (10-fold or 10x dilution). Be sure to mix the bottles thoroughly before and after the sample is added.
Refer to Sample Dilution in the Introduction to Bacteria section to find the number of dilutions that must be made for the sample type. For example if Class A sludge, add a 100X sample dilution to five tubes, a 1000X sample dilution to five tubes and a 10,000X sample dilution to five tubes. If the coliform density is not known, add five different dilutions to 5 sets of MPN tubes.
If all the tubes are positive, dilute the sample several more times and repeat the test. If all tubes are negative, the sample was diluted too many times. Repeat the test with less serial dilutions.
If more than three dilutions were made, use three consecutive dilutions that contain both positive and negative tubes.
The dilution factor for an undiluted sample is 1.
No confirmation is needed when using A-1 Medium.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

Collect the following items:

Description	Quantity
A-1 Medium Broth Tubes	15
Dilution Water, buffered, 99-mL, sterile	3 bottles
Incubator	1
Pipet, serological, 10-11 mL, sterile	3
Pipet filler	1
Coliform tube rack	1

See *Consumables and replacement items* for reorder information.

Fecal Coliforms, A-1 Medium Broth, method 8368

1. Wash thoroughly with soap and water.

Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed.

2. Using sterile buffered dilution water, prepare at least 3 serial dilutions of the sample.

Refer to *Sample dilution* for instructions.

3. Remove the caps from 15 tubes of A-1 Medium Broth. Use a sterile pipet to transfer 10 mL of the first dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the second dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the third dilution into the remaining 5 tubes.

Do not touch the open end of the tubes or the inside of the caps.

4. Replace the screw cap on each tube immediately after the sample is added. Invert and swirl the tube several times to thoroughly mix the sample with the nutrient medium. After the last inversion, make sure that the inner vial is full of liquid with no air bubbles.

5. Place the tubes in the incubator at a temperature of 35 ±0.5 °C.

Any bubbles that form in the inner vials during this period are not from bacteria. Be sure to remove the bubbles by inverting the tubes. Make sure there are no bubbles and then carefully return the tubes to an upright position. Return the tubes to the incubator.

6. After 3 hours, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator.

Increase the temperature to 44.5 ±0.2 °C and incubate for an additional 21 hours. The tubes must be kept upright for the rest of the test.

7. After 24 ±2 hours, tap each tube gently and examine the inner vials for gas. If the inner vials contain gas bubbles, the test is positive for fecal coliform bacteria. Count the number of tubes that contain gas.

If none of the tubes contain gas, the test is negative for fecal coliform bacteria.

8. Find the MPN index per 100 mL from the *MPN index table*.

Multiply the MPN index by the dilution factor in the first set of tubes (lowest dilution used in the test).

(MPN index x lowest dilution factor = fecal coliform bacteria per 100 mL sample)

See the *Example calculation*.

Sample dilution

Complete the following procedure to make serial dilutions of the sample. Refer to the *Dilution guidelines by sample type* to find the number of times the sample must be diluted. Use the three dilutions from the *Dilution guidelines by sample type* for the test (step 3 of the fecal coliform procedure).

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container for 30 seconds, approximately 25 times.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert the sample (for 30 seconds) 25 times. This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until there are three bottles that contain the dilutions listed in the *Dilution guidelines by sample type*.

   Note: *Shaking the sample too vigorously will injure or stress the organisms.*

Table 376 Dilution guidelines by sample type

Sample type	Dilution 1	Dilution 2	Dilution 3
Swimming pool water, chlorinated	undiluted (1x)	10x	100x
Bathing beach water	10x	100x	1000x
Lake water	10x	100x	1000x
Unpolluted river water	10x	100x	1000x
Final effluent, chlorinated	100x	1000x	10,000x
River water, polluted	1000x	10,000x	100,000x
Storm water	10,000x	100,000x	1,000,000x
Unchlorinated final effluent	10,000x	100,000x	1,000,000x
Raw sewage	100,000x	1,000,000x	10,000,000x

Example calculation

A sample was diluted into 3 different buffered dilution bottles. The dilutions were 10-fold (10x), 100-fold (100x) and 1000-fold (1000x). 5 MPN tubes were filled from each dilution (15 tubes total). The first set of tubes (10x) had four tubes with gas, the second set (100x) had 2 tubes with gas and the third set (1000x) had 1 tube with gas.

1. Find the MPN index for the three sets of tubes from the *MPN index table*.
2. Multiply the MPN index by the lowest dilution factor.

The MPN index from the *MPN index table* table for 4, 2 and 1 positive tubes is 26. The coliform result for the sample is 26 x 10 = 260 coliforms per 100 mL of sample.

Table 377 MPN index table

Number of positive tubes			MPN index per 100 mL	Number of positive tubes			MPN index per 100 mL
First dilution set	Second dilution set	Third dilution set		First dilution set	Second dilution set	Third dilution set	
0	0	0	< 2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	30
1	1	0	4	5	0	2	40
1	1	1	6	5	1	0	30
1	2	0	6	5	1	1	50
2	0	0	4	5	1	2	60
2	0	1	7	5	2	0	50
2	1	0	7	5	2	1	70
2	1	1	9	5	2	2	90
2	2	0	9	5	3	0	80
2	3	0	12	5	3	1	110
3	0	0	8	5	3	2	140
3	0	1	11	5	3	3	170
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
4	0	0	13	5	4	4	350
4	0	1	17	5	5	0	240
4	1	0	17	5	5	1	300
4	1	1	21	5	5	2	500
4	1	1	26	5	5	3	900
4	2	0	22	5	5	4	1600
				5	5	5	≥ 1600

Sample collection, preservation and storage

- Collect at least 100 mL of sample in sterilized Whirl-Pak® bags, sterilized disposable bottles or autoclaved glass or plastic bottles.
- Do not fill sample containers completely. Leave at least 2.5 cm (approximately 1 inch) of air space to allow adequate space for mixing the sample prior to analysis.
- Make sure that the samples are representative of the sample source. Fill sample containers from a tank or reservoir entirely under water.
- Start the analysis as soon as possible after collection. Allow no more than 6 hours to elapse after collection. If the test cannot be started immediately, cool the sample to below 10 °C. Do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

Controls

Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device. Potable water samples from municipal treatment facilities should be negative for total and fecal coliforms.

Bacteria disposal

To safely dispose of bacterial cultures left in the broth tubes, use one of the following methods:

Bleach
Sterilize used test containers with household bleach. Add 1–2 mL of the bleach to each test container. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.

Autoclave
Place used test containers in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test containers in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. When cool, seal the bag, place it in another garbage bag and tie tightly.

Summary of method

The Most Probable Number (MPN) method (also referred to as the Multiple Tube Fermentation Technique) uses screw-capped tubes containing sterile broth medium. The tubes contain an inverted inner vial (Durham tube) for gas collection. Sample is diluted, added to the tubes and incubated. If coliforms are present, gas is produced and is trapped in the inner vial.

The number of tubes that form gas is used to estimate the number of coliform organisms in the sample. The MPN method allows for the analysis of highly turbid samples by dilution prior to analysis. No filtering is necessary.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
A-1 Medium Broth Tubes	15/pkg	2560915
Dilution Water, buffered, 99-mL, sterile[1]	25/pkg	1430598

[1] Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Required apparatus

Description	Unit	Catalog number
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	100/pkg	2233199
Incubator, 12-well Dri-Bath, 120 VAC, 50/60 Hz	each	2281400
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Pipet, serological, 10-11 mL, sterile, disposable	25/pkg	209798
Pipet safety bulb	each	1465100

Optional media and reagents

Description	Unit	Catalog number
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

Optional apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, with dechlorinating agent, 180-mL	100/pkg	2075333
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	500/pkg	2233100
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, polysulfone, autoclavable (use for buffered dilution water)	12/pkg	2245300
Bottle, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Germicidal Cloths	50/pkg	2463200
Incubator, Portable, 12 VDC	each	2569900
Marker, Laboratory	each	2092000
Pipet, Serological, 1-mL, sterile, disposable, individually wrapped	50/pkg	2092835
Pipet, Serological, 10-mL, sterile, disposable, individually wrapped	50/pkg	2092628
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, sterile, individually wrapped	200/pkg	2558996
Pipet Aid, 110 VAC Recharger, 4 replacement filters (UL, CSA approved)	each	2551701
Rack, Coliform Tube	each	221500

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Edition 7

Coliforms—Total and *E. Coli*

DOC316.53.01218

Lauryl Tryptose with MUG Broth[1]

Method 8091

Most Probable Number (MPN) Method

Scope and Application: For potable and non-potable water.

[1] Based on publication by Peter C.S. Feng and Paul A. Hartman "Fluorogenic Assays for Immediate Confirmation of Escherichia coli". *Applied and Environmental Microbiology*, Vol. 43, No. 6, pp. 1320-1329, 1982. This method is not USEPA accepted.

Before starting the test:

Make sure that all materials used for containing or transferring samples are sterile.
The bottles of dilution water contain 99 mL of sterile buffered dilution water. When 11 mL of sample is added to a 99-mL bottle of dilution water, the sample is diluted by a factor of 10 (10-fold or 10x dilution). Be sure to mix the bottles thoroughly before and after the sample is added.
If all the tubes are positive, dilute the sample several more times and repeat the test. If all tubes are negative, the sample was diluted too many times. Repeat the test with less serial dilutions.
If more than three dilutions were made, use three consecutive dilutions that contain both positive and negative tubes.
The dilution factor for an undiluted sample is 1.
Fluorescence without gas production indicates an anaerogenic (non-gas-producing) strain(s) of *E. coli*.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray, or dilute iodine solution. Wash hands thoroughly with soap and water.

Collect the following items:

Description	Quantity
Lauryl Tryptose with MUG Broth Tubes	5–15
Dilution Water, buffered, 99-mL, sterile (for non-potable water samples only)	3 bottles
Coliform tube rack	1
Incubator	1
Pipet, serological, 10-11 mL, sterile	3
Pipet filler	1

See *Consumables and replacement items* for reorder information.

Potable water test for coliforms—total and *E. coli*, Lauryl Tryptose with MUG Broth

1. Wash thoroughly with soap and water.

Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed.

2. Remove the caps from 5 or 10 tubes of Lauryl Tryptose with MUG Broth one at a time. Use a sterile pipet to transfer 10 mL of sample into each of the tubes.

Do not touch the open end of the tubes or the inside of the caps.

3. Replace the screw cap on each tube immediately after the sample is added. Invert the tube several times to thoroughly mix the sample with the nutrient medium. After the last inversion, make sure the inner vial is full of liquid with no air bubbles.

4. Place the tubes in the incubator at a temperature of 35 (±0.5) °C.

5. After one hour, invert the tubes to remove trapped air in the inner vials.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure there are no bubbles and then carefully return the tubes to an upright position.

Loosen the caps slightly before returning the tubes to the incubator. Continue incubation. The tubes must be kept upright for the rest of the test.

6. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely present.

The presence of gas in any amount is an indication of coliform bacteria.

If none of the tubes contain gas, the test is negative for total coliform bacteria.

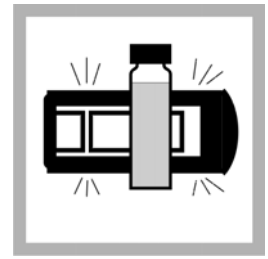
If tubes are cloudy but have no gas bubbles, check the tubes for fluorescence (step 7).

7. Use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

If the tube does not fluoresce, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

Compare the fluorescence of the sample tubes to a tube containing a known *E. coli* culture to make a positive confirmation.



8. After 48 (±3) hours, use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

If there is no fluorescence, the test is negative for *E. coli*.

Refer to *Potable water MPN results* to find the MPN of the sample.

Non-potable water test for coliforms—total and *E. coli*, Lauryl Tryptose with MUG Broth

1. Wash thoroughly with soap and water.

Invert the sample for 30 seconds, approximately 25 times, to make sure that it is well-mixed.

2. Using sterile buffered dilution water, prepare at least 3 serial dilutions of the sample.

Refer to *Sample dilution* for instructions.

3. Remove the caps from 15 tubes of Lauryl Tryptose with MUG Broth. Use a sterile pipet to transfer 10 mL of the first dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the second dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the third dilution into the remaining 5 tubes.

Do not touch the open end of the tubes or the inside of the caps.

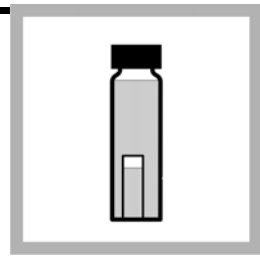
4. Replace the screw cap on each tube immediately after the sample is added. Invert the tube several times to thoroughly mix the sample with the nutrient medium. After the last inversion, make sure the inner vial is full of liquid with no air bubbles.

Non-potable water test for coliforms—total and *E. coli*, Lauryl Tryptose with MUG Broth (continued)

5. Place the tubes in the incubator at a temperature of 35 ±0.5 °C.

After one hour, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator.

Continue incubation. The tubes must be kept upright for the rest of the test.

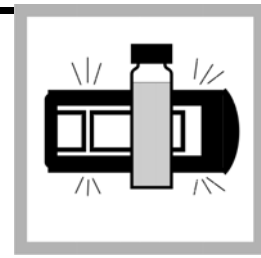


6. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely present.

The presence of gas in any amount is an indication of coliform bacteria.

If none of the tubes contain gas, the test is negative for total coliform bacteria.

If tubes are cloudy but have no gas bubbles, check the tubes for fluorescence (step 7).

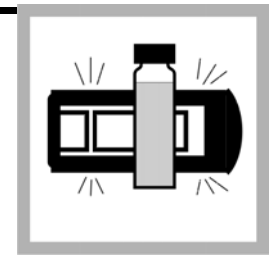


7. Use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

If the tube does not fluoresce, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

Compare the fluorescence of the sample tubes to a tube containing a known *E. coli* culture to make a positive confirmation.



8. After 48 (±3) hours, use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

If the solution shows fluorescence, the test is positive for *E. coli*.

If there is no fluorescence, the test is negative for E. coli.

Refer to *Non-potable water MPN results* to find the MPN of the sample.

Sample dilution

Complete the following procedure to make serial dilutions of the sample. Refer to the *Dilution guidelines by sample type* to find the number of times the sample must be diluted. Use the three dilutions from the *Dilution guidelines by sample type* for the test.

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container for 30 seconds, approximately 25 times.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert (for 30 seconds) 25 times. This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until there are three bottles that contain the dilutions listed in the *Dilution guidelines by sample type*.

Note: *Shaking the sample too vigorously will injure or stress the organisms.*

Table 378 Dilution guidelines by sample type

Sample type	Dilution 1	Dilution 2	Dilution 3
Swimming pool water, chlorinated	undiluted (1x)	10x	100x
Bathing beach water	10x	100x	1000x
Lake water	10x	100x	1000x
Unpolluted river water	10x	100x	1000x
Final effluent, chlorinated	100x	1000x	10,000x
River water, polluted	1000x	10,000x	100,000x
Storm water	10,000x	100,000x	1,000,000x
Unchlorinated final effluent	10,000x	100,000x	1,000,000x
Raw sewage	100,000x	1,000,000x	10,000,000x

Potable water MPN results

Use the number of positive tubes to find the MPN per 100 mL from the *MPN table for 10 tubes*.

Example: 6 of the 10 tubes showed a positive response. The MPN per 100 mL is 9.2.

Table 379 MPN table for 10 tubes[1]

Number of positive tubes	MPN per 100 mL
0	< 1.1
1	1.1
2	2.2
3	3.6
4	5.1
5	6.9
6	9.2
7	12.0
8	16.1
9	23.0
10	> 23.0

[1] Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits.

5 broth tubes can be used in place of 10 tubes and the *MPN table for 5 tubes* can be used.

Table 380 MPN table for 5 tubes[1]

Number of positive tubes	MPN per 100 mL
0	< 2.2
1	2.2
2	5.1
3	9.2
4	16.0
5	> 16.0

[1] Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits.

Non-potable water MPN results

A sample was diluted into 3 different buffered dilution bottles. The dilutions were 10-fold (10x), 100-fold (100x) and 1000-fold (1000x). 5 MPN tubes were filled from each dilution (15 tubes total). The first set of tubes (10x) had four tubes with gas, the second set (100x) had 2 tubes with gas and the third set (1000x) had 1 tube with gas.

1. Find the MPN index for the three sets of tubes from the *MPN index table*.
2. Multiply the MPN index by the lowest dilution factor.

The MPN index from the *MPN index table* table for 4, 2 and 1 positive tubes is 26. The coliform result for the sample is 26 x 10 = 260 coliforms per 100 mL of sample.

Table 381 MPN index table

Number of positive tubes			MPN index per 100 mL	Number of positive tubes			MPN index per 100 mL
First dilution set	Second dilution set	Third dilution set		First dilution set	Second dilution set	Third dilution set	
0	0	0	< 2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	30
1	1	0	4	5	0	2	40
1	1	1	6	5	1	0	30
1	2	0	6	5	1	1	50
2	0	0	4	5	1	2	60
2	0	1	7	5	2	0	50
2	1	0	7	5	2	1	70
2	1	1	9	5	2	2	90
2	2	0	9	5	3	0	80
2	3	0	12	5	3	1	110
3	0	0	8	5	3	2	140
3	0	1	11	5	3	3	170
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
4	0	0	13	5	4	4	350
4	0	1	17	5	5	0	240
4	1	0	17	5	5	1	300
4	1	1	21	5	5	2	500
4	1	1	26	5	5	3	900
4	2	0	22	5	5	4	1600
				5	5	5	≥ 1600

Sample collection, preservation and storage

- Collect at least 100 mL of sample in sterilized Whirl-Pak® bags, sterilized disposable bottles or autoclaved glass or plastic bottles.
- Do not fill sample containers completely. Leave at least 2.5 cm (approximately 1 inch) of air space to allow adequate space for mixing the sample prior to analysis.
- Make sure that the samples are representative of the sample source. Fill sample containers from a tank or reservoir entirely under water.
- Start the analysis as soon as possible after collection. Allow no more than 6 hours to elapse after collection. If the test cannot be started immediately, cool the sample to below 10 °C. Do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

Controls

Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Bacteria disposal

To safely dispose of bacterial cultures left in the broth tubes, use one of the following methods:

Bleach
Sterilize used test containers with household bleach. Add 1–2 mL of the bleach to each test container. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.

Autoclave
Place used test containers in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test containers in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. When cool, seal the bag, place it in another garbage bag, and tie tightly.

Summary of method

The Most Probable Number (MPN) method (also referred to as the Multiple Tube Fermentation Technique) uses screw-capped tubes containing sterile broth medium. The tubes contain an inverted inner vial (Durham tube) for gas collection. Sample is diluted, added to the tubes and incubated. If coliforms are present, gas is produced and is trapped in the inner vial.

The number of tubes that form gas is used to estimate the number of coliform organisms in the sample. The MPN method allows for the analysis of highly turbid samples by dilution prior to analysis. No filtering is necessary.

The Lauryl Tryptose with MUG Broth will detect total coliforms and *E. coli*. The results are comparable to the traditional MPN fecal coliform tests, but obtained in far less time. No transfer from presumptive to confirmed medium is necessary with the LT/MUG method. The LT/MUG medium contains lauryl tryptose broth and 4-methylumbelliferyl-ß-D-glucuronide (MUG), a fluorogenic reagent. Tubes positive for *E. coli* will fluoresce when the incubated tubes are examined under long-wave UV light.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Lauryl Tryptose with MUG Broth Tubes	15/pkg	2182115
Dilution Water, buffered, 99-mL, sterile	25/pkg	1430598

Required apparatus

Description	Unit	Catalog number
Bags, Whirl-Pak®, with dechlorinating agent, 180-mL	100/pkg	2075333
Incubator, 12-well Dri-Bath, 120 VAC, 50/60 Hz	each	2281400
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	each	2184302
Pipet, serological, 10-11 mL, sterile, disposable	25/pkg	209798
Pipet safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 720-mL	100/pkg	1437297
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	500/pkg	2233100
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Brilliant Green Bile (BGB) Broth Tubes	15/pkg	32215
Bottle, polysulfone, autoclavable (use for buffered dilution water)	12/pkg	2245300
Bottle, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bottle, sample, sterilized, 100-mL fill-to line, disposable w/dechlorinating agent	50/pkg	2599150
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Ecoli Fluorescence standard	each	2361100
Germicidal Cloths	50/pkg	2463200
Incubator, Portable, 12 VDC	each	2569900
Marker, Laboratory	each	2092000
Pipet, Serological, 1-mL, sterile, disposable, individually wrapped	50/pkg	2092835
Pipet, Serological, 10-mL, sterile, disposable, individually wrapped	50/pkg	2092628
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, sterile, individually wrapped	200/pkg	2558996
Pipet Aid, 110 VAC Recharger, 4 replacement filters (UL, CSA approved)	each	2551701

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Rack, Coliform Tube	each	221500
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Coliforms—Total, Fecal and *E. Coli* DOC316.53.01217

USEPA[1] Lauryl Tryptose Broth presumptive test with BGB, EC Medium and EC/MUG confirmation

Method 8001A

Most Probable Number (MPN) Method

Scope and Application: For non-potable water and wastewater.

[1] Most Probable Number Method 8001A for wastewater is USEPA-accepted. Method 8001A meets or exceeds the specification criteria stated in *Standard Methods for the Examination of Water and Wastewater*, 19th edition, 9221 Multiple-Tube Fermentation Technique for Members of the Coliform Group.

Before starting the test:

Make sure that all the materials that are used for containing or transferring samples are sterile.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Use the Lauryl Tryptose Broth for the presumptive test. If the presumptive test is positive, use Brilliant Green Bile (BGB) broth to confirm if the sample has total coliforms, EC Medium for fecal coliforms or EC Medium with MUG for *E. coli*. The confirmation test is used to eliminate false-positive results that can occur with the presumptive test.
The bottles of dilution water contain 99 mL of sterile buffered dilution water. When 11 mL of sample is added to a 99-mL bottle of dilution water, the sample is diluted by a factor of 10 (10-fold or 10x dilution). Be sure to mix the bottles thoroughly before and after the sample is added.
If all the tubes are positive, dilute the sample several more times and repeat the test. If all tubes are negative, the sample was diluted too many times. Repeat the test with less serial dilutions.
For USEPA reporting, the confirmation tubes must be inoculated by an inoculation loop. Cap transfer is not allowed.
If more than three dilutions were made, use three consecutive dilutions that contain both positive and negative tubes.
The dilution factor for an undiluted sample is 1.
To sterilize an inoculating needle, heat the needle to red hot in an alcohol or Bunsen burner. Let the needle cool before use.

Collect the following items:

Description	Quantity
Lauryl Tryptose Broth Tubes	15
Brilliant Green Bile (BGB) Broth Tubes	varies
EC Medium Broth Tubes	varies
EC Medium with MUG Broth Tubes	varies
Dilution Water, buffered, 99-mL, sterile	3 or more bottles
Incubator	1
Alcohol burner	1
Inoculating loop	1
Pipet, serological, 10–11 mL, sterile	1
Pipet filler	1
Coliform tube rack	1

See *Consumables and replacement items* for reorder information.

Presumptive test for coliform bacteria (Lauryl Tryptose Broth)

1. Wash thoroughly with soap and water.

Invert the sample for 30 seconds, approximately 25 times, to make sure that it is well-mixed.

2. Using sterile buffered dilution water, prepare at least 3 serial dilutions of the sample.

Refer to *Sample dilution* for instructions.

3. Remove the caps from 15 tubes of Lauryl Tryptose Broth one at a time. Use a sterile pipet to transfer 10 mL of the first dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the second dilution into 5 tubes. Use a sterile pipet to transfer 10 mL of the third dilution into the remaining 5 tubes.

Do not touch the open end of the tubes or the inside of the caps.

4. Replace the screw cap on each tube immediately after the sample is added. Invert the tube several times to thoroughly mix the sample with the nutrient medium. After the last inversion, make sure the inner vial is full of liquid with no air bubbles.

5. Place the tubes in the incubator at a temperature of 35 (±0.5) °C.

Any bubbles that form in the inner vials during the first hour are not from bacteria.

6. After one hour, invert the tubes to remove trapped air in the inner vials.

Remove the bubbles by inverting the tubes. Make sure there are no bubbles and then carefully return the tubes to an upright position.

Loosen the caps slightly before returning the tubes to the incubator. Continue incubation. The tubes must be kept upright for the rest of the test.

7. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely present. If no gas can be seen, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

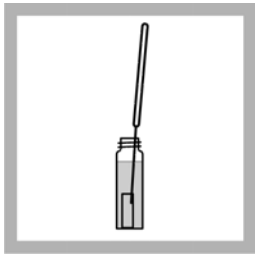
The presence of gas in any amount is an indication of coliform bacteria.

8. Count the number of tubes that contain gas in the inner vial.

Complete a confirmation test for all tubes that contain gas. The confirmation test will confirm whether total coliforms, fecal coliforms or E.Coli are present in the sample.

If none of the tubes contain gas, the test is negative for coliform bacteria.

Confirmation test for total coliforms (Brilliant Green Bile Broth)



1. From each positive LT Broth tube, inoculate a Brilliant Green Bile (BGB) Broth tube. Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a BGB Broth tube, making sure not to touch the rim of either tube.

2. Replace the screw cap on each tube immediately. Invert the BGB tubes to remove trapped air in the inner vials.

3. Place the BGB tubes in the incubator at a temperature of 35 (±0.5) °C.

4. After one hour, invert the tubes to remove trapped air in the inner vials.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure the bubbles are gone and then carefully return the tubes to an upright position.

Loosen the caps slightly before returning the tubes to the incubator. Continue incubation. The tubes must be kept upright for the rest of the test.

5. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for total coliform bacteria.

If no gas can be seen, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

6. After 48 (±3) hours, tap each tube gently and examine the inner vials for gas. If the inner vials contain gas bubbles, the test is positive for total coliform bacteria. Count the number of tubes that contain gas.

If none of the tubes contain gas, the test is negative for coliforms.

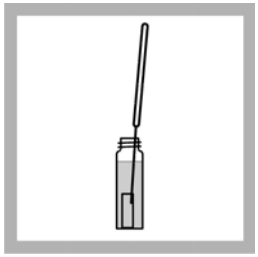
7. Find the MPN index per 100 mL from the *MPN index table*.

Multiply the MPN index by the dilution factor in the first set of tubes (lowest dilution used in the test).

(MPN index x lowest dilution factor = total coliform bacteria per 100 mL sample)

See the *Example calculation*.

Confirmation test for fecal coliforms (EC Medium)



1. From each positive LT Broth tube, inoculate an EC Medium Broth tube.

Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a EC Medium tube, making sure not to touch the rim of either tube.

2. Replace the screw cap on each tube immediately. Invert the EC Medium Broth tubes to remove trapped air in the inner vials.

3. Place the EC Medium Broth tubes in the incubator at a temperature of 44.5 (±0.2) °C.

4. After one hour, invert the tubes to remove trapped air in the inner vials.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure the bubbles are gone and then carefully return the tubes to an upright position.

Loosen the caps slightly before returning the tubes to the incubator. Continue incubation. The tubes must be kept upright for the rest of the test.

5. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for fecal coliform bacteria. Count the number of tubes that contain gas.

If none of the tubes contain gas, the test is negative for fecal coliforms.

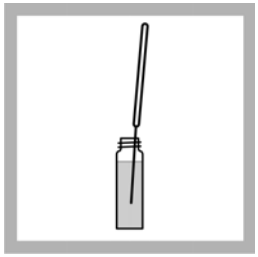
6. Find the MPN index per 100 mL from the *MPN index table*.

Multiply the MPN index by the dilution factor in the first set of tubes (lowest dilution used in the test).

(MPN index x lowest dilution factor = fecal coliform bacteria per 100 mL sample)

See the *Example calculation*.

Confirmation test for *E. coli* (EC Medium with MUG)



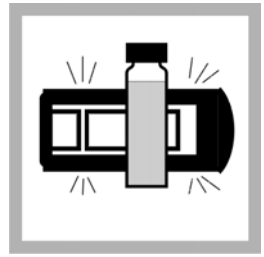
1. From each positive LT Broth tube, inoculate an EC Medium with MUG Broth tube.

Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a EC Medium with MUG tube, making sure not to touch the rim of either tube.

Replace the screw cap on each tube immediately. Invert to mix.

2. Place the EC Medium with MUG tubes in the incubator at a temperature of 44.5 (±0.2) °C for 24 (±2) hours.



3. After 24 (±2) hours, use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

Compare the fluorescence of the sample tubes to a tube containing a known *E. coli* culture to make a positive confirmation.

If the solution shows fluorescence, the test is positive for *E. coli*. If there is no fluorescence, the test is negative for *E. coli*.

4. Count the number of tubes that show fluorescence.

Find the MPN index per 100 mL from the *MPN index table*.

Multiply the MPN index by the dilution factor in the first set of tubes (lowest dilution used in the test).

(MPN index x lowest dilution factor = *E. coli* bacteria per 100 mL sample)

See the *Example calculation*.

Sample collection, preservation and storage

- Collect at least 100 mL of sample in sterilized Whirl-Pak® bags, sterilized disposable bottles or autoclaved glass or plastic bottles.
- Do not fill sample containers completely. Leave at least 2.5 cm (approximately 1 inch) of air space to allow adequate space for mixing the sample prior to analysis.
- Make sure that the samples are representative of the sample source. Fill sample containers from a tank or reservoir entirely under water.
- Start the analysis as soon as possible after collection. Allow no more than 6 hours to elapse after collection. If the test cannot be started immediately, cool the sample to below 10 °C. Do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

Note: *If more than 6 hours will elapse after collection, refer to the preservation and storage requirements in Standard Methods for the Examination of Water and Wastewater.*

Bacteria disposal

To safely dispose of bacterial cultures left in the broth tubes, use one of the following methods:

Bleach
Sterilize used test tubes with household bleach. Add 1–2 mL of the bleach to each test tube. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.

Autoclave
Place used test tubes in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test tubes in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. When cool, seal the bag, place it in another garbage bag and tie tightly.

Sample dilution

Complete the following procedure to make serial dilutions of the sample. Refer to the *Dilution guidelines by sample type* to find the number of times the sample must be diluted. Use the three dilutions from the *Dilution guidelines by sample type* for the test (step 3 of the *Presumptive test for coliform bacteria (Lauryl Tryptose Broth)*).

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container for 30 seconds, approximately 25 times.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert (for 30 seconds) 25 times. This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until there are three bottles that contain the dilutions listed in the *Dilution guidelines by sample type*.

Note: *Shaking the sample too vigorously will injure or stress the organisms.*

Table 382 Dilution guidelines by sample type

Sample type	Dilution 1	Dilution 2	Dilution 3
Swimming pool water, chlorinated	undiluted (1x)	10x	100x
Bathing beach water	10x	100x	1000x
Lake water	10x	100x	1000x
Unpolluted river water	10x	100x	1000x
Final effluent, chlorinated	100x	1000x	10,000x
River water, polluted	1000x	10,000x	100,000x
Storm water	10,000x	100,000x	1,000,000x
Unchlorinated final effluent	10,000x	100,000x	1,000,000x
Raw sewage	100,000x	1,000,000x	10,000,000x

Example calculation

A sample was diluted into 3 different buffered dilution bottles. The dilutions were 10-fold (10x), 100-fold (100x) and 1000-fold (1000x). 5 MPN tubes were filled from each dilution (15 tubes total). The first set of tubes (10x) had four tubes with gas, the second set (100x) had 2 tubes with gas and the third set (1000x) had 1 tube with gas.

1. Find the MPN index for the three sets of tubes from the *MPN index table*.
2. Multiply the MPN index by the lowest dilution factor (e.g. 10 for a 10x dilution).

The MPN index from the *MPN index table* table for 4, 2 and 1 positive tubes is 26. The coliform result for the sample is 26 x 10 = 260 coliforms per 100 mL of sample.

Table 383 MPN index table

Number of positive tubes			MPN index per 100 mL	Number of positive tubes			MPN index per 100 mL
Dilution 1	Dilution 2	Dilution 3		Dilution 1	Dilution 2	Dilution 3	
0	0	0	< 2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	30
1	1	0	4	5	0	2	40
1	1	1	6	5	1	0	30
1	2	0	6	5	1	1	50
2	0	0	4	5	1	2	60
2	0	1	7	5	2	0	50
2	1	0	7	5	2	1	70
2	1	1	9	5	2	2	90
2	2	0	9	5	3	0	80
2	3	0	12	5	3	1	110
3	0	0	8	5	3	2	140
3	0	1	11	5	3	3	170
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
4	0	0	13	5	4	4	350
4	0	1	17	5	5	0	240
4	1	0	17	5	5	1	300
4	1	1	21	5	5	2	500
4	1	1	26	5	5	3	900
4	2	0	22	5	5	4	1600
				5	5	5	≥ 1600

Controls

Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device. Potable water samples from municipal treatment facilities should be negative for total and fecal coliforms.

Summary of method

The Most Probable Number (MPN) method (also referred to as the Multiple Tube Fermentation Technique) uses screw-capped tubes containing sterile broth medium. The tubes contain an inverted inner vial (Durham tube) for gas collection. Sample is added to the tubes and incubated. If coliforms are present, gas is produced and is trapped in the inner vial. The number of tubes that form gas is used to estimate the number of coliform organisms in the sample. When the EC Medium with MUG broth is used, fluorescence under a longwave UV lamp confirms the presence of *E. coli*.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Lauryl Tryptose Broth Tubes	15/pkg	2101415
Brilliant Green Bile (BGB) Broth Tubes	15/pkg	32215
EC Medium Broth Tubes	15/pkg	1410415
EC Medium with MUG Broth Tubes	15/pkg	2471515
Dilution Water, buffered, 99-mL, sterile	25/pkg	1430598

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	each	2087742
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	100/pkg	2233199
Incubator, 12-well Dri-Bath, 120 VAC, 50/60 Hz	each	2281400
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Inoculating Loop, nichrome wire	each	2112100
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	each	2184302
Pipet, serological, 10-11 mL, sterile, disposable	25/pkg	209798
Pipet safety bulb	each	1465100

Optional reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, with dechlorinating agent, 180-mL	100/pkg	2075333
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	500/pkg	2233100
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
E. coli Fluorescence standard	each	2361100
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Inoculating Loops, sterile, disposable	25/pkg	2749125
Isopropyl alcohol	500 mL	1445949
Lamp, long-wave, ultraviolet, portable, 4 watt	each	2415200

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Marker, laboratory	each	2092000
Pipet, serological, 1-mL, sterile, disposable, individually wrapped	50/pkg	2092835
Pipet, serological, 10-mL, sterile, disposable, individually wrapped	50/pkg	2092628
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, sterile, individually wrapped	200/pkg	2558996
Pipet Aid, 110 VAC recharger, 4 replacement filters (UL, CSA approved)	each	2551701
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Rack, coliform tube	each	221500
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Wicks, replacement, for alcohol burner 2087742		2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

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Edition 7

Coliforms—Total, Fecal and *E. Coli* DOC316.53.01216

USEPA[1] Lauryl Tryptose Broth presumptive test with BGB, EC Medium and EC/MUG confirmation

Method 8001

Most Probable Number (MPN) Method

Scope and Application: For potable water.

[1] Most Probable Number Method 8001 for potable water is USEPA-accepted. Method 8001 meets or exceeds the specification criteria stated in Standard Methods for the Examination of Water and Wastewater, 19th edition, 9221 Multiple-Tube Fermentation Technique for Members of the Coliform Group. For potable water, confirm fecal coliforms with EC Medium Broth as cited in 40 CFR Part 141.21, Subpart (F)(5); or confirm *E. coli* with EC/MUG Medium Broth as cited in 40 CFR Part 141.21, Subpart (F)(6)(i).

Before starting the test:

Make sure that all materials that are used for containing or transferring samples are sterile.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Use the Lauryl Tryptose Broth for the presumptive test. If the presumptive test is positive, use Brilliant Green Bile (BGB) broth to confirm if the sample has total coliforms, then EC Medium for fecal coliforms or EC Medium with MUG for *E. coli*.
Potable water should not contain any coliform bacteria. Samples should not be diluted.
For USEPA reporting, the confirmation tubes must be inoculated by an inoculation loop. Cap transfer is not allowed.
To sterilize an inoculating needle, heat the needle to red hot in an alcohol or Bunsen burner. Let the needle cool before use.
If all 10 tubes (for a 10-tube MPN test) of the confirmed coliform test are negative, the sample is accepted as meeting bacterial standards. To make sure that sample results are interpreted in accordance with appropriate standards and regulations, contact the local, county, state or federal regulatory agency.
If the test will not be used for USEPA reporting, 5 broth tubes can be used in place of 10 tubes. Use the *MPN table for 5 tubes* to find the result of the 5-tube test. The 5-tube test cannot be used for USEPA reporting.

Collect the following items:

Description	Quantity
Lauryl Tryptose Broth Tubes	10
Brilliant Green Bile (BGB) Broth Tubes	varies
EC Medium Broth Tubes	varies
EC Medium with MUG Broth Tubes	varies
Incubator	1
Alcohol burner	1
Inoculating loop	1
Pipet, serological, 10–11 mL, sterile	1
Pipet filler	1
Coliform tube rack	1

See *Consumables and replacement items* for reorder information.

Presumptive test for coliform bacteria (Lauryl Tryptose Broth)

1. Wash thoroughly with soap and water.

Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed.

2. Remove the caps from 10 tubes of Lauryl Tryptose Broth one at a time. Use a sterile pipet to transfer 10 mL of sample into each of the tubes.

Do not touch the open end of the tubes or the inside of the caps.

3. Replace and tighten the screw cap on each tube immediately after the sample is added. Invert and swirl the tube several times to thoroughly mix the sample with the nutrient medium. After the last inversion, make sure the inner vial is full of liquid with no air bubbles.

4. Place the tubes in the incubator at a temperature of 35 (±0.5) °C.

5. After one hour, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator.

Continue incubation. The tubes must be kept upright for the rest of the test.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure there are no bubbles and then carefully return the tubes to an upright position.

6. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely present. If no gas can be seen, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

The presence of gas in any amount is an indication of coliform bacteria.

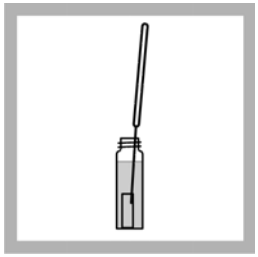
Count the number of tubes that contain gas in the inner vial.

7. Complete a confirmation test for all tubes that contain gas. The confirmation test will confirm whether total coliforms, fecal coliforms or E.Coli are present in the sample.

The confirmation test is used to eliminate false-positive results that can occur with the presumptive test.

If none of the tubes contain gas, the test is negative for coliform bacteria.

Confirmation test for total coliforms (Brilliant Green Bile Broth)



1. From each positive LT Broth tube, inoculate a Brilliant Green Bile (BGB) Broth tube. Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a BGB Broth tube, making sure not to touch the rim of either tube.

2. Replace and tighten the screw cap on each tube immediately. Invert and swirl the BGB tubes to remove trapped air in the inner vials.

If Durham tube vials are used, after the last inversion, make sure that the inner tube does not contain any air bubbles.

3. Place the BGB tubes in the incubator at a temperature of 35 (±0.5) °C.

4. After one hour, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator.

Continue incubation. The tubes must be kept upright for the rest of the test.

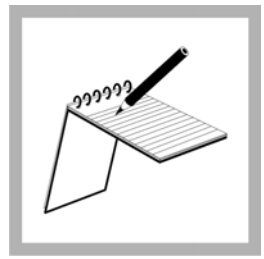
Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure the bubbles are gone and then carefully return the tubes to an upright position.

5. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for total coliform bacteria.

If no gas can be seen, return the tubes to the incubator and examine again after a total of 48 (±3) hours.

6. After 48 (±3) hours, tap each tube gently and examine the inner vials for gas. If the inner vials contain gas bubbles, the test is positive for total coliform bacteria.

If none of the tubes contain gas, the test is negative for coliform bacteria.

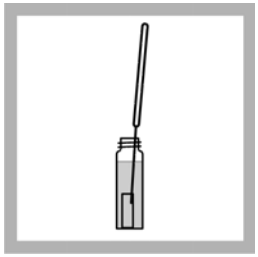


7. Count the number of tubes that contain gas in the inner vial.

Find the MPN of the sample (total coliform bacteria per 100 mL sample) from the *MPN table for 10 tubes*.

8. If the test is positive for total coliform bacteria, complete a confirmation test for fecal coliform or E.Coli bacteria (USEPA requirement).

Confirmation test for fecal coliforms (EC Medium)



1. From each positive LT Broth tube, inoculate an EC Medium Broth tube.

Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a EC Medium tube, making sure not to touch the rim of either tube.

2. Replace and tighten the screw cap on each tube immediately. Invert and swirl the EC Medium Broth tubes to remove trapped air in the inner vials.

If Durham tube vials are used, after the last inversion, make sure that the inner tube does not contain any air bubbles.

3. Place the EC Medium Broth tubes in the incubator at a temperature of 44.5 (±0.2) °C.

4. After one hour, invert the tubes to remove trapped air in the inner vials. Loosen the caps slightly before returning the tubes to the incubator.

Continue incubation. The tubes must be kept upright for the rest of the test.

Any bubbles that form in the inner vials during the first hour are not from bacteria. Remove the bubbles by inverting the tubes. Make sure the bubbles are gone and then carefully return the tubes to an upright position.

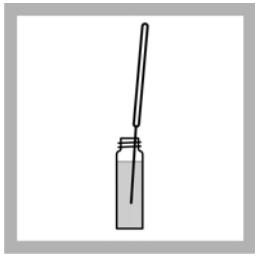
5. After 24 (±2) hours, tap each tube gently and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for fecal coliform bacteria.

If none of the tubes contain gas, the test is negative for fecal coliform bacteria.

6. Count the number of tubes that contain gas in the inner vial.

Find the MPN of the sample (fecal coliform bacteria per 100 mL sample) from the *MPN table for 10 tubes*.

Confirmation test for *E. coli* (EC Medium with MUG)



1. From each positive LT Broth tube, inoculate an EC Medium with MUG Broth tube.

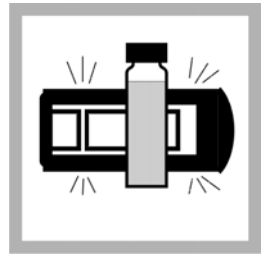
Use a sterile, disposable loop or a flame-sterilized, nichrome wire.

Put the loop into the positive Lauryl Tryptose tube and then into a EC Medium with MUG tube, making sure not to touch the rim of either tube.

Replace and tighten the screw cap on each tube immediately. Invert and swirl to mix.

If Durham tube vials are used, after the last inversion, make sure that the inner tube does not contain any air bubbles.

2. Place the EC Medium with MUG tubes in the incubator at a temperature of 44.5 (±0.2) °C for 24 (±2) hours.



3. After 24 (±2) hours, use a longwave ultraviolet (UV) lamp to check the tubes for fluorescence. Examine the tubes in a dark area.

Compare the fluorescence of the sample tubes to a tube containing a known *E. coli* culture to make a positive confirmation.

If the solution shows fluorescence, the test is positive for *E. coli*. If there is no fluorescence, the test is negative for *E. coli*.

4. Count the number of tubes that show fluorescence.

Find the MPN of the sample (*E. coli* bacteria per 100 mL sample) from the *MPN table for 10 tubes*.

Sample collection, preservation and storage

- Collect at least 100 mL of sample in sterilized Whirl-Pak® bags, sterilized disposable bottles or autoclaved glass or plastic bottles.
- Do not fill sample containers completely. Leave at least 2.5 cm (approximately 1 inch) of air space to allow adequate space for mixing the sample prior to analysis.
- Make sure that the samples are representative of the sample source. Fill sample containers from a tank or reservoir entirely under water.
- Start the analysis as soon as possible after collection. Allow no more than 30 hours to elapse after collection. If the test cannot be started immediately, cool the sample to below 10 °C. Do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

MPN table

Use the number of positive tubes to find the MPN per 100 mL from the *MPN table for 10 tubes*.

Example: 6 of the 10 tubes showed a positive response. The MPN per 100 mL is 9.2.

Table 384 MPN table for 10 tubes[1]

Number of positive tubes	MPN per 100 mL
0	< 1.1
1	1.1
2	2.2
3	3.6
4	5.1
5	6.9
6	9.2
7	12.0
8	16.1
9	23.0
10	> 23.0

[1] Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits.

If the test will not be used for USEPA reporting, 5 broth tubes can be used in place of 10 tubes and the *MPN table for 5 tubes* can be used. The 5-tube test cannot be used for USEPA reporting.

Table 385 MPN table for 5 tubes[1]

Number of positive tubes	MPN per 100 mL
0	< 2.2
1	2.2
2	5.1
3	9.2
4	16.0
5	> 16.0

[1] Table is for undiluted samples, 10 mL per tube. Values are 95 percent confidence limits. The MPN table for 5 tubes cannot be used for USEPA reporting.

Bacteria disposal

To safely dispose of bacterial cultures left in the broth tubes, use one of the following methods:

Bleach
Sterilize used test tubes with household bleach. Add 1–2 mL of the bleach to each test tube. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.

Autoclave
Place used test tubes in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test tubes in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. When cool, seal the bag, place it in another garbage bag and tie tightly.

Summary of method

The Most Probable Number (MPN) method (also referred to as the Multiple Tube Fermentation Technique) uses screw-capped tubes containing sterile broth medium. The tubes contain an inverted inner vial (Durham tube) for gas collection. Sample is added to the tubes and incubated. If coliforms are present, gas is produced and is trapped in the inner vial. The number of tubes that form gas is used to estimate the number of coliform organisms in the sample. When the EC Medium with MUG broth is used, fluorescence under a longwave UV lamp confirms the presence of *E. coli*.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Lauryl Tryptose Broth tubes	15/pkg	2101415
Brilliant Green Bile (BGB) Broth tubes	15/pkg	32215
EC Medium Broth tubes	15/pkg	1410415
EC Medium with MUG Broth tubes (without Durham tubes)	15/pkg	2471515
EC Medium with MUG Broth tubes (with Durham tubes)	15/pkg	2282415

Required apparatus

Description	Unit	Catalog number
Alcohol Burner	each	2087742
Bags, Whirl-Pak®, with dechlorinating agent, 170-mL	100/pkg	2075333
Incubator, 12-well Dri-Bath, 120 VAC, 50/60 Hz	each	2281400
Inoculating Loop, nichrome wire	each	2112100
Lamp, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, long-wave, ultraviolet, 230 VAC, 50/60 Hz	each	2184302
Pipet, serological, 10-11 mL, sterile, disposable	25/pkg	209798
Pipet safety bulb	each	1465100

Optional media and reagents

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dilution Water, buffered, 99-mL, sterile	25/pkg	1430598
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

Optional apparatus

Description	Unit	Catalog number
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 207-mL	500/pkg	2233100
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700
E. coli Fluorescence standard	each	2361100
Germicidal Cloths	50/pkg	2463200
Inoculating Loops, sterile, disposable	25/pkg	2749125
Isopropyl alcohol	500 mL	1445949
Lamp, long-wave, ultraviolet, portable, 4 watt	each	2415200
Marker, laboratory	each	2092000
Pipet, serological, 1-mL, sterile, disposable, individually wrapped	50/pkg	2092835
Pipet, serological, 10-mL, sterile, disposable, individually wrapped	50/pkg	2092628
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, sterile, individually wrapped	200/pkg	2558996
Pipet Aid, 110 VAC recharger, 4 replacement filters (UL, CSA approved)	each	2551701
Rack, coliform tube	each	221500
Wicks, replacement, for alcohol burner 2087742		2097810

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Edition 7

Bacteria, Hydrogen Sulfide Producing

DOC316.53.01196

Most Probable Number (MPN) Method **Method 10032**

Pathoscreen™ Medium

Scope and Application: For the detection of Salmonella, Citrobacter, Proteus, Edwardsiella and Klebsiella (some spp.) in drinking water, surface water and recreational water

Test preparation

PathoScreen Medium

PathoScreen Medium detects the presence of hydrogen sulfide-producing bacteria including *Salmonella, Citrobacter, Proteus, Edwardsiella and* some species of *Klebsiella*. The sterilized powder medium is a reliable medium for monitoring drinking water systems in developing tropical countries, in remote field locations and in disaster or emergency situations.

PathoScreen Medium is dehydrated, sterilized and packaged in powder pillows. Powder pillows are available for both Presence/Absence (P/A) and Most Probable Number (MPN) testing. Each powder pillow contains enough medium for one test. The medium is shipped with a Certificate of Analysis and has an expiration date printed on the label.

For MPN testing, add one MPN Pillow to a 20-mL sample. For MPN testing, inoculate a set of five tubes.

Conducting MPN tests with PathoScreen Medium

The MPN method can be used for drinking water, as well as marine and fresh recreational waters, swimming pools, lakes, shellfish-growing waters, heavily polluted waters and wastewater. For water that is heavily contaminated, use the multiple tube decimal dilution procedure.

Before starting the test:

Incubate samples 24–48 hours between 25–35 °C, 77–95 °F. (30 °C, 80 °F is considered optimal.)
PathoScreen Medium has a detection sensitivity of 1 CFU/100 mL.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution.
Wash hands thoroughly with soap and water.

PathoScreen medium MPN pillows, method 10032

1. Wash hands thoroughly with soap and water.

2. Remove the caps from five sterile tubes one at a time and pipet 20 mL of sample into each of the tubes with a sterile pipet. Use aseptic technique to avoid contaminating the tubes or the caps.

3. Swab the end of a PathoScreen Medium MPN Pillow with alcohol and aseptically cut it open with clippers. Add pillow contents to the 20 mL sample.

4. Cap and swirl each tube and immediately. Invert the tubes a few times to thoroughly mix the sample with the medium. The sample will be yellow in color.

5. Place the tubes in a location with a constant temperature of 25–35 °C for 24–48 hours.
6. If an incubator is available, incubate the sample at 30 ± 0.5 °C for 24–48 hours.

7. Note the reaction after 24 hours of incubation. (See the *MPN Results* table.)

If the temperature varies significantly, continue to incubate negative tubes for an additional day.

8. Record results. (See the *Five-tube MPN values* table.)

Dispose of all completed tests appropriately. Refer to the MSDS and local regulations for proper disposal.

Table 386 MPN Results

Hydrogen sulfide producing bacteria			
Test Results	**Positive**	**Negative**	**Follow-up**
Color changes from yellow to black	X		
Black precipitate forms	X		
No color change		X	Incubate additional 12–24 hours and re-evaluate. If there is no color change, record as negative.

Using statistical methods it is possible to estimate the number of organisms from any combination of positive and negative test results. The MPN values in the *Five-tube MPN values* table are based on 20 mL of undiluted sample in each of five tubes. If the sample is diluted, multiply the result by the dilution factor.

Example 1:
Five tubes of undiluted sample are inoculated. Positive results are obtained from three of the five tubes. The result obtained from the *Five-tube MPN values* table is 4.6.

Example 2:
A river water sample is collected and diluted. A dilution factor of 10,000 is prepared and five tubes are inoculated. Positive results are obtained from two of the five tubes. The result obtained from two of the five tubes. The result obtained from the *Five-tube MPN values* table is 2.6. This result is multiplied by 10,000 and a result of 26,000 is recorded.

Table 387 Five-tube MPN values

Positive Tubes	MPN/100 mL
0	<1.1
1	1.1
2	2.6
3	4.6
4	8.0
5	>8.0

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
PathoScreen™ Medium, MPN Pillows, 20-mL sample	50/pkg	2610796

Dilution water—media, reagents and apparatus

Description	Unit	Catalog number
Buffered Dilution Water, sterile, 99-mL[1]	25/pkg	1430598
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Pipet, sterile, disposable, 11-mL	25/pkg	209798
Pipet, sterile, disposable, individually wrapped, 10-mL	50/pkg	2092628
Pipet, sterile, disposable, 10-mL	50/pkg	2092828
Pipet Filler, portable, with recharger (UL, CSA approved), 110 VAC	each	2551701

[1] Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Required apparatus

Description	Unit	Catalog number
Autoclave, Automatic, 120 VAC	each	2898600
Clippers, large	each	2065800
Contaminated Items Bags	200/pkg	2463300
Germicidal Cloth	50/pkg	2463200
Incubator, Culture, 120 VAC	each	2619200
Incubator, Culture, 220 VAC	each	2619202
MPN Vials	10/pkg	1497054
Rack for coliform tubes	each	2497903
Sampling Containers		
Sampling Bags, Whirl-Pak with dechlorinating agent, 180-mL	100/pkg	2075333
Sampling Bottles, autoclavable	6/pkg	2324333
Sampling Bottles, autoclavable	48/pkg	2324373
Sampling Bottles, sterilized, 100-mL fill-to line	12/pkg	2495012
Sampling Bottles, sterilized, 100-mL fill-to line	50/pkg	2495050
Sampling Bottles, sterilized, 100-mL fill-to line, with dechlorinating agent	12/pkg	2599112
Sampling Bottles, sterilized, 100-mL fill-to line, with dechlorinating agent	50/pkg	2599150

Optional media, reagents and apparatus

Description	Unit	Catalog number
Bottle, polysulfone, autoclavable (for preparing buffered dilution water)	12/pkg	2245300
Magnesium Chloride and Potassium Dihydrogen Phosphate Powder Pillows	25 of each	2143166
Peptone Powder Pillows, 1-g	30/pkg	2142964
Safety bulb	each	1465100
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon	100/pkg	2811199
Bags, Whirl-Pak®, without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak, without dechlor 720 mL	10/pkg	1437297
Incubator, 12-well, Dri Bath	each	2281400
Pipet, TenSette® 1.0–10.0 mL	each	1970010
Pipet tips, sterile, individually wrapped	200/pkg	2558996

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Edition 7

Coliforms

DOC316.53.01191

Presence/Absence **Methods 8319 and 8364**

P/A Broth (8319)
P/A Broth w/ MUG (8364)

Scope and Application: For water and wastewater

Test preparation

Before starting the test:

Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray, or dilute iodine solution
Wash hands thoroughly with soap and water.

Introduction

Both P/A Broth and P/A Broth with MUG come packaged in disposable bottles and in glass ampules. The medium in both bottles and ampules is concentrated and must be diluted with sterile water 5:1. The medium is sterilized by membrane filtration to prevent degradation.

P/A Broth ampules, method 8319

1. Collect 100 mL of sample in a sterile sample container. Use aseptic technique to avoid contaminating the sample and sample container.

2. Add sample to the fill-to line of a sample bottle.

Sample may be added from a sterile container, or directly from a faucet or spigot.

3. Add the contents of one P/A Broth ampule to the 100 mL of sample.

4. Incubate the sample at 35 ± 0.5 °C for 24–48 hours.

P/A Broth ampules, method 8319

5. Note the reaction after 24 hours of incubation. See *Interpret and report results on page 1383*.

6. Confirm presumptive positive samples by inoculating the appropriate media directly from positive P/A broth cultures. See the *Confirmation Table on page 1383*.

7. Dispose of all completed tests appropriately.

Dispose of all completed tests

P/A broth with MUG, method 8364

1. Collect 100 mL of sample in a sterile sample container. Use aseptic technique to avoid contaminating the sample and sample container.

2. Add sample to the fill-to line of a Broth Disposable Bottle.

Sample may be added from a sterile container, or directly from a faucet or spigot.

3. Incubate the sample at 35 ±0.5 °C for 24–48 hours.

4. Note the reaction after 24 hours of incubation. See *Interpret and report results*.

P/A broth with MUG, method 8364 (continued)

5. Confirm presumptive positive samples by inoculating the appropriate media directly from positive P/A broth cultures. See the *Confirmation Table*.

6. Dispose of all completed tests appropriately.

Interpret and report results

Reactions using P/A Broth

- *Color change from reddish purple to yellow or yellow brown*—record the test as presumptive positive for total coliform bacteria.
- *No color change*—incubate for an additional 24 hours and recheck the sample for color change.

  If after 24 hours of incubation, the *color changes from reddish purple to yellow or yellow brown*—record the test as presumptive positive for total coliform bacteria.

  If after 48 ±3 hours of incubation, the sample *still appears reddish purple*—record the test as negative for total coliform bacteria.

Reactions using P/A Broth with MUG

- Examine the sample under long-wave UV light; *if the sample fluoresces*—record the test as positive for *E. coli*.

Confirm positive samples

Inoculum from incubated presence/absence samples are used to confirm the presence of total coliforms, fecal coliforms or *E. coli* in the original water sample. Use a sterile inoculating loop to transfer sample to an appropriate confirmation medium.

Table 388 Confirmation Table

Bacteria	Confirmation Medium	Incubation	Incubator	Positive Result
Total Coliform	Brilliant Green Bile Broth (322-15)	24 to 48 hours. 35 ± 0.5 °C	Culture (2619200 or -02) or 12-well Dri-Bath (2281400)	Gas/Turbidity
Fecal Coliform	EC Medium Tubes (14104-15)	24 hours 44.5 ± 0.2 °C	12-well Dri-Bath (2281400)	Gas/Turbidity
E. coli	EC Medium with MUG Tubes (24715-15)	24 hours 44.5 ± 0.2 °C	12-well Dri-Bath (2281400)	Fluorescence

Controls

Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device. Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

Completed test disposal

Active bacterial cultures grown during incubation must be disposed of safely. Use either *Bleach* or an *Autoclave* to neutralize bacteria.

Bleach

1. Sterilize used test containers with a 10% bleach solution.
2. Add approximately 12 mL of bleach to each test containerand allow to stand for 10 to 15 minutes.
3. Pour the liquid down the drain. Dispose of the test tubes with the normal garbage.

Autoclave
Test containers must be placed in a bag before autoclaving to prevent leakage of solution into the autoclave.

1. Place used test tubes in a contaminated-items bag or a biohazard bag and seal tightly.
1. Autoclave the used test tubes in the bag at 121 °C for 15 minutes at 15 pounds of pressure.
2. Place the bag of test tubes in a separate garbage bag and tie tightly.
3. Once the test tubes are sterile, dispose of them with the normal garbage.

Summary of method 8319

P/A Broth is ideal for screening drinking water samples for total coliforms. The method is a simple modification of the multiple-tube method. It uses lactose and lauryl tryptose broths with bromcresol purple, which detects acidity formed during lactose fermentation by the bacteria.

Simply combine 100 mL of sample and P/A Broth, incubate for 24 hours and check for a color change. A yellow color indicates the presence of total coliforms.

Summary of method 8364

P/A Broth with MUG allows simultaneous detection of total coliform bacteria and *E. coli*. In addition to the lactose and lauryl tryptose broths with bromcresol purple, this medium contains MUG reagent (4-methylumbelliferyl-b-D-glucuronide). The MUG reagent produces a fluorogenic product when hydrolyzed by glucuronidase (an enzyme specific to E. coli). MUG detects non gas-producing (anaerogenic) strains of *E. coli* and works well when competitive organisms are present.

Combine 100 mL of sample and P/A Broth with MUG, incubate for 24 hours and check for a color change and fluorescence. A yellow color indicates the presence of total coliforms. To detect *E. coli*, examine samples under a long-wave ultraviolet (UV) light. Fluorescence indicates the presence of *E. coli*.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
P/A Broth Ampules		
P/A Broth Ampules	25/pkg	2494925
P/A Broth with MUG Ampules	25/pkg	2495525
P/A Broth in Disposable Bottles		
P/A Broth Disposable Bottles	12/pkg	2323212
P/A Broth Disposable Bottles	50/pkg	2323250
P/A Broth with MUG Disposable Bottles	12/pkg	2401612
P/A Broth with MUG Disposable Bottles	50/pkg	2401650

Required apparatus

Description	Unit	Catalog number
Bottles, sample, presterilized, 100-mL fill-to line	12/pkg	2495012
Incubator, Culture, low profile, 110/120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220/240 VAC, 50/60 Hz	each	2619202
Lamp, handheld, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, handheld, long-wave, ultraviolet, 230 VAC, 50 Hz	each	2184302

Optional apparatus

Description	Unit	Catalog number
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bags for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®[1], with dechlorinating agent	100/pkg	2075333
Bottles, sample, presterilized, 100-mL fill-to line	50/pkg	2495050
Bottles, sample, presterilized, with dechlorinating agent, 100-mL fill-to line	12/pkg	2599112
Bottles, sample, presterilized, with dechlorinating agent, 100-mL fill-to line	12/pkg	2599150
Lamp, long-wave, ultraviolet, portable	each	2415200
Incubator, Dri-Bath, 12-Well, 120V	each	2281400
Bags, Whirl-Pak[1], without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak[1], without dechlor 720 mL	10/pkg	1437297
Dechlorinating reagent, powder pillows	100/pkg)	1436369
Inoculating loop, nichrome V wire	each	2112100
Inoculating loop, disposable	25/pkg	2749125
Isopropyl alcohol	500 mL	1445949
Alcohol burner	each	2087742
Replacement wicks for 2087742	each	2097810
Incubator, portable, 12 VDC	each	2569900
Battery eliminator	each	2580400

Optional apparatus

Description	Unit	Catalog number
Rechargeable battery pack, for portable incubator 12V DC / 115 V AC adapter	each	2580300
230V AC Rechargeable batter pack adapter (for 2580300)	rach	2595902

[1] Whirl-Pak is a registered trademark of Nasco, Inc.

Optional media

Description	Unit	Catalog number
Bottles, sample, presterilized, 100-mL fill-to line	12/pkg	2495012
Incubator, Culture, low profile, 110/120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, low profile, 220/240 VAC, 50/60 Hz	each	2619202
Lamp, handheld, long-wave, ultraviolet, 115 VAC, 60 Hz	each	2184300
Lamp, handheld, long-wave, ultraviolet, 230 VAC, 50 Hz	each	2184302

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Edition 7

Bacteria, Hydrogen Sulfide Producing

DOC316.53.01197

Presence/Absence (P/A) Method **Methods 8506**

Pathoscreen™ Medium

Scope and Application: For the detection of Salmonella, Citrobacter, Proteus, Edwardsiella and Klebsiella (some spp.) in drinking water, surface water and recreational water

Test preparation

PathoScreen Medium

PathoScreen Medium detects the presence of hydrogen sulfide-producing bacteria including *Salmonella, Citrobacter, Proteus, Edwardsiella and* some species of *Klebsiella*. The sterilized powder medium is a reliable medium for monitoring drinking water systems in developing tropical countries, in remote field locations and in disaster or emergency situations.

PathoScreen Medium is dehydrated, sterilized and packaged in powder pillows. Powder pillows are available for both Presence/Absence (P/A) and Most Probable Number (MPN) testing. Each powder pillow contains enough medium for one test. The medium is shipped with a Certificate of Analysis and has an expiration date printed on the label.

For P/A testing, add one P/A powder pillow to a 100-mL sample.

Before starting the test:

Incubate samples 24–48 hours between 25–35 °C, 77–95 °F. (30 °C, 80 °F is considered optimal.)
PathoScreen Medium has a detection sensitivity of 1 CFU/100 mL.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution.
Wash hands thoroughly with soap and water.

PathoScreen medium P/A pillows, method 8506

1. Wash hands thoroughly with soap and water.

2. Collect 100 mL of sample in a sterile sample container.

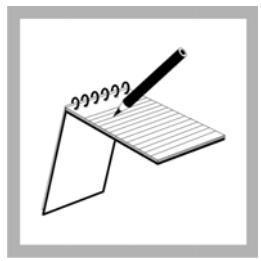
3. Swab the end of the PathoScreen Medium P/A Pillow with alcohol and aseptically cut it open with clippers. Add pillow contents to the 100 mL sample.

4. Place the bottle in a location with a constant temperature between 25–35 °C for 24–48 hours.

If an incubator is available, incubate the sample at 30 ±0.5 °C for 24 to 48 hours.

5. Note the reaction after 24 hours of incubation.

If the temperature varies significantly, incubation may be extended an additional day.



6. Record the results. (See the *P/A results* table.)

Dispose of all completed tests appropriately. Refer to a current MSDS and local regulations.

Table 389 P/A results

Hydrogen sulfide producing bacteria			
Test Results	**Positive**	**Negative**	**Follow-up**
Color changes from yellow to black	X		
Black precipitate forms	X		
No color change		X	Incubate additional 12–24 hours and re-evaluate. If there is no color change, record as negative.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
PathoScreen™ Medium, P/A Pillows, 100-mL sample	50/pkg	2610696

Dilution water—media, reagents and apparatus

Description	Unit	Catalog number
Bottle, polysulfone, autoclavable (for preparing buffered dilution water)	12/pkg	2245300
Buffered Dilution Water, sterile, 99-mL[1]	25/pkg	1430598
Dechlorinating Reagent Powder Pillows	100/pkg	1436369

[1] Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Required apparatus

Description	Unit	Catalog number
Autoclave, Automatic, 120 VAC	each	2898600
Clippers, large	each	2065800
Contaminated Items Bags	200/pkg	2463300
Germicidal Cloth	50/pkg	2463200
Incubator, Culture, 120 VAC	each	2619200
Incubator, Culture, 220 VAC	each	2619202
Sampling Containers		
Sampling Bags, Whirl-Pak with dechlorinating agent, 180-mL	100/pkg	2075333
Sampling Bottles, autoclavable	6/pkg	2324333
Sampling Bottles, autoclavable	48/pkg	2324373
Sampling Bottles, sterilized, 100-mL fill-to line	12/pkg	2495012
Sampling Bottles, sterilized, 100-mL fill-to line	50/pkg	2495050
Sampling Bottles, sterilized, 100-mL fill-to line, with dechlorinating agent	12/pkg	2599112
Sampling Bottles, sterilized, 100-mL fill-to line, with dechlorinating agent	50/pkg	2599150

Optional media and reagents

Description	Unit	Catalog number
Bottle, polysulfone, autoclavable (for preparing buffered dilution water)	12/pkg	2245300
Magnesium Chloride and Potassium Dihydrogen Phosphate Powder Pillows	25 of each	2143166
Peptone Powder Pillows, 1-g	30/pkg	2142964
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon	100/pkg	2811199
Bags, Whirl-Pak®, without dechlor 207 mL	100/pkg	2233199
Bags, Whirl-Pak, without dechlor 720 mL	10/pkg	1437297

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Edition 7

Heterotrophic Bacteria

DOC316.53.01225

Pour Plate Method **Method 8242**

m-HPC

Scope and Application: For water and wastewater.

Test preparation

Introduction

The Pour Plate Method, also known as the standard plate count, is simple to perform and is commonly used to determine heterotrophic bacteria density. This method does, however, have disadvantages that limit recovery of the maximum number of organisms. Tempered medium at 44–46 °C (111–115 °F) may cause heat shock to stressed bacteria and the nutritionally rich medium may decrease recovery of starved bacteria.

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultatively anaerobic heterotrophic bacteria in water. Bacteria occur singly or in pairs, chains, clusters or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. However, the heterotrophic plate count is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of source water.

Before starting the test:

See the *Introduction to Bacteria* for more information about preparing sample containers and collecting and preserving samples.
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.
Limit the number of samples to be plated at any one time so that no more than 20 minutes (preferably 10 minutes) elapse between the dilution of the first sample and the pouring of the last plate.
To save time, start the incubator before preparing the other materials. Set the incubator for the temperature required in the procedure (usually 35 ± 0.5 °C).
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Mark each pour plate, membrane filtration petri dish, or other sample container with the sample number, dilution, date, and any other necessary information. Take care not to contaminate the inside of the sample container in any way.

Pour plate procedure for heterotrophic bacteria m-HPC, method 8242

1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish. **Do not touch the pad or the inside of the petri dish.**

To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

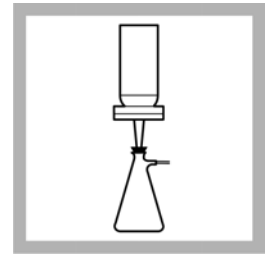
Alternatively, a prepared m-HPC agar plate may be used.

2. Invert ampules two or three times to mix broth. Open an ampule of m-HPC Broth, using an ampule breaker if necessary. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

For broth prepared from dehydrated medium, pipet approximately 2.0 mL of broth onto the pad using a sterile pipet. Drain excess medium from the petri dish and replace the lid.

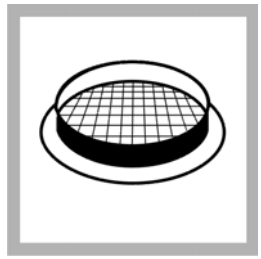
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter, grid side up, in the assembly.

Alternatively, a sterile, disposable filter unit may be used.



4. Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed. Filter the appropriate volume through the sterile 47 mm, 0.45µm, gridded membrane filter. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20–30 mL of sterile buffered dilution water.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ±0.5 °C for 48 hours.

8. Remove the dish from the incubator. Count colonies on membrane filters using a 10–15X microscope.

Bacterial colonies grown on m-HPC medium appear clear to cream in color.

Diluting the Sample

The pour plate method requires use of 1 mL, 0.1 mL, and 0.01 mL or 0.001 mL of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 mL, require the use of sample dilutions. These dilutions are prepared by pipetting 1 mL of undiluted sample into 99 mL of buffered dilution water. Diluting the sample allows 1 mL of diluted sample to be used instead of 0.01 mL of undiluted sample, and 0.1 mL of diluted sample instead of 0.001 mL of undiluted sample.

Selecting Sample Volumes/Dilutions

Select the sample volumes or dilutions to be used so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL of undiluted sample, 0.1 mL of undiluted sample and 1 mL of diluted sample (which equals 0.01 mL of undiluted sample). In examining sewage or turbid water, do not measure a 0.1-mL inoculum of the original undiluted sample, but do prepare an appropriate dilution.

Counting, Computing and Reporting Results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time, and the medium.

For example: 98 CFU/L, mL, 35 °C, 24 hours, m-HPC broth.

1 to 2, or fewer colonies per square — Count all of the colonies on the filter, and divide the results by the volume of original sample used.
For example, if there are 122 colonies on the filter, and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{\text{122 colonies}}{\text{10 mL sample}} = \text{12.2 CFU/mL}$$

3 to 10 colonies per square — Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.
For example, if you calculated an average of 8 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{8 colonies/square x 100}}{\text{0.1 mL sample}} = \text{8000 CFU/mL}$$

10 to 20 colonies per square — Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.

For example: if there are an average of 17 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{17 colonies/square x 100}}{\text{0.1 mL sample}} = \text{17, 000 CFU/mL}$$

More than 20 colonies per square—If there are more than 20 colonies per square, record the count as > 2000 divided by the volume of original sample used.
For example, if the original volume of sample used were 0.01 mL, results would be > 2000/0.01 or > 200,000 CFU/mL.

Note: *Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.*

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Dilution Water, Buffered, sterile, 99-mL	25/pkg	1430598
m-HPC Agar Plates	15/pkg	2811415
m-HPC Broth Ampules, plastic, 2-mL	50/pkg	2812450

Required apparatus

Description	Unit	Catalog number
Absorbent Pads with dispenser, sterile, Gelman	1000/pkg	1491800
Ampule Breaker, PourRite™	each	2484600
Whirl-Pak Bags with declorinating agent, sterile, 180-mL	100/pkg	2075333
Filter Holder, magnetic coupling	each	1352900
Filtering Flask, 500-mL	each	54649
Forceps	each	2141100
Incubator, Culture, low profile, 110 VAC	each	2619200
Incubator, Culture, low profile, 220 VAC	each	2619202
Membrane filters, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Membrane filters, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Microscope, Compound	each	2942500
Petri Dish, polystyrene, sterile, disposable, without pad	100/pkg	1485299
Petri Dish, polystyrene, sterile, disposable, w/pad, Gelman	100/pkg	1471799
Petri Dish, polystyrene, sterile, disposable, w/pad, Millipore	150/pkg	2936300
Pump, vacuum, 110/115 VAC	each	2824800
Pump, vacuum, 220/230 VAC, Continental European Plug	each	2824802
Rubber Stopper, one hole, No. 8	6/pkg	211908
Rubber Tubing, 3.6-m	each	56019
Pipets, Serological, 10–11 mL, sterile, disposable	25/pkg	209798

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	each	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Counter, hand tally	each	1469600
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Isopropyl alcohol	500 mL	1445949
Microscope, Stereo Binocular	each	2942600
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Wicks, replacement, for alcohol burner 2087742	—	2097810

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Edition 7

Heterotrophic Bacteria

DOC316.53.01227

Pour Plate Method **Method 8242**

m-TGE with TTC

Scope and Application: For water and wastewater.

Test preparation

Introduction

The Pour Plate Method, also known as the standard plate count, is simple to perform and is commonly used to determine heterotrophic bacteria density. This method does, however, have disadvantages that limit recovery of the maximum number of organisms. Tempered medium at 44–46 °C (111–115 °F) may cause heat shock to stressed bacteria and the nutritionally rich medium may decrease recovery of starved bacteria.

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultatively anaerobic heterotrophic bacteria in water. Bacteria occur singly or in pairs, chains, clusters or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. However, the heterotrophic plate count is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of source water.

Before starting the test:

See the *Introduction to Bacteria* for more information about preparing sample containers and collecting and preserving samples.
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.
Limit the number of samples to be plated at any one time so that no more than 20 minutes (preferably 10 minutes) elapse between the dilution of the first sample and the pouring of the last plate.
To save time, start the incubator before preparing the other materials. Set the incubator for the temperature required in the procedure (usually 35 ± 0.5 °C).
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Mark each pour plate, membrane filtration petri dish, or other sample container with the sample number, dilution, date, and any other necessary information. Take care not to contaminate the inside of the sample container in any way.

Pour plate procedure for heterotrophic bacteria m-TGE with TTC, method 8242

1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish. **Do not touch the pad or the inside of the petri dish.**

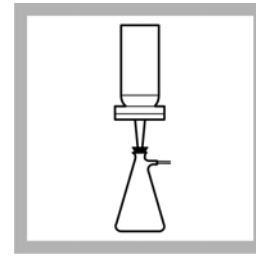
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

2. Invert ampules two or three times to mix broth. Open an ampule of m-TGE with TTC or use an ampule breaker if necessary. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

For broth prepared from dehydrated medium, pipet approximately 2.0 mL of broth onto the pad using a sterile pipet. Drain excess medium from the petri dish and replace the lid.

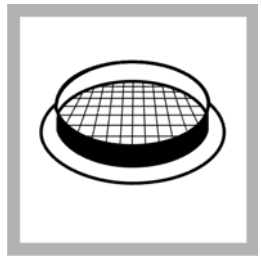
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter, grid side up, in the assembly.

Alternatively, a sterile, disposable filter unit may be used.



4. Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed. Filter the appropriate volume through the sterile 47 mm, 0.45µm, gridded membrane filter. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20–30 mL of sterile buffered dilution water.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ± 0.5 °C for 24 hours.

8. Remove the dish from the incubator. Count colonies on membrane filters using a 10–15X microscope.

Bacterial colonies grown on m-TGE with TTC medium appear red to aid visibility.

Diluting the Sample

The pour plate method requires use of 1 mL, 0.1 mL, and 0.01 mL or 0.001 mL of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 mL, require the use of sample dilutions. These dilutions are prepared by pipetting 1 mL of undiluted sample into 99 mL of buffered dilution water. Diluting the sample allows 1 mL of diluted sample to be used instead of 0.01 mL of undiluted sample, and 0.1 mL of diluted sample instead of 0.001 mL of undiluted sample.

Selecting Sample Volumes/Dilutions

Select the sample volumes or dilutions to be used so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL of undiluted sample, 0.1 mL of undiluted sample and 1 mL of diluted sample (which equals 0.01 mL of undiluted sample). In examining sewage or turbid water, do not measure a 0.1-mL inoculum of the original undiluted sample, but do prepare an appropriate dilution.

Counting, Computing and Reporting Results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time, and the medium.

For example: 98 CFU/L, mL, 35 °C, 24 hours, m-TGE with TTC broth.

1 to 2, or fewer colonies per square—Count all of the colonies on the filter, and divide the results by the volume of original sample used.
For example, if there are 122 colonies on the filter, and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{\text{122 colonies}}{\text{10 mL sample}} = 12.2 \text{ CFU/mL}$$

3 to 10 colonies per square — Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.
For example, if you calculated an average of 8 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{8 colonies/square x 100}}{\text{0.1 mL sample}} = 8000 \text{ CFU/mL}$$

10 to 20 colonies per square—Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.

For example: if there are an average of 17 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{17 colonies/square x 100}}{\text{0.1 mL sample}} = 17,000 \text{ CFU/mL}$$

More than 20 colonies per square—If there are more than 20 colonies per square, record the count as > 2000 divided by the volume of original sample used.
For example, if the original volume of sample used were 0.01 mL, results would be > 2000/0.01 or > 200,000 CFU/mL.

Note: *Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.*

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Dilution Water, Buffered, sterile, 99-mL	25/pkg	1430598
m-TGE with TTC PourRite™ Ampules, glass, 2-mL	20/pkg	2428420

Required apparatus

Description	Unit	Catalog number
Absorbent Pads with dispenser, sterile, Gelman	1000/pkg	1491800
Ampule Breaker, PourRite™	each	2484600
Whirl-Pak Bags with declorinating agent, sterile, 180-mL	100/pkg	2075333
Filter Holder, magnetic coupling	each	1352900
Filtering Flask, 500-mL	each	54649
Forceps	each	2141100
Incubator, Culture, low profile, 110 VAC	each	2619200
Incubator, Culture, low profile, 220 VAC	each	2619202
Membrane filters, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Membrane filters, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Microscope, Compound	each	2942500
Petri Dish, polystyrene, sterile, disposable, without pad	100/pkg	1485299
Petri Dish, polystyrene, sterile, disposable, w/pad, Gelman	100/pkg	1471799
Petri Dish, polystyrene, sterile, disposable, w/pad, Millipore	150/pkg	2936300
Pump, vacuum, 110/115 VAC	each	2824800
Pump, vacuum, 220/230 VAC, Continental European Plug	each	2824802
Rubber Stopper, one hole, No. 8	6/pkg	211908
Rubber Tubing, 3.6-m	each	56019
Pipets, Serological, 10–11 mL, sterile, disposable	25/pkg	209798

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	each	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Counter, hand tally	each	1469600
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Isopropyl alcohol	500 mL	1445949
Microscope, Stereo Binocular	each	2942600
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Wicks, replacement, for alcohol burner 2087742	—	2097810

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Edition 7

Heterotrophic Bacteria

DOC316.53.01226

Pour Plate Method **Method 8242**

m-TGE

Scope and Application: For water and wastewater.

Test preparation

Introduction

The Pour Plate Method, also known as the standard plate count, is simple to perform and is commonly used to determine heterotrophic bacteria density. This method does, however, have disadvantages that limit recovery of the maximum number of organisms. Tempered medium at 44–46 °C (111–115 °F) may cause heat shock to stressed bacteria and the nutritionally rich medium may decrease recovery of starved bacteria.

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultatively anaerobic heterotrophic bacteria in water. Bacteria occur singly or in pairs, chains, clusters or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. However, the heterotrophic plate count is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of source water.

Before starting the test:

See the *Introduction to Bacteria* for more information about preparing sample containers and collecting and preserving samples.
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.
Limit the number of samples to be plated at any one time so that no more than 20 minutes (preferably 10 minutes) elapse between the dilution of the first sample and the pouring of the last plate.
To save time, start the incubator before preparing the other materials. Set the incubator for the temperature required in the procedure (usually 35 ± 0.5 °C).
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Mark each pour plate, membrane filtration petri dish, or other sample container with the sample number, dilution, date, and any other necessary information. Take care not to contaminate the inside of the sample container in any way.

Pour plate procedure for heterotrophic bacteria m-TGE, method 8242

1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish. **Do not touch the pad or the inside of the petri dish.**

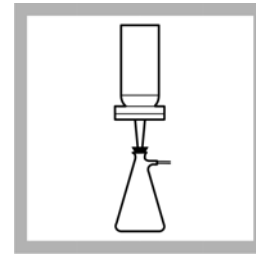
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

2. Invert ampules two or three times to mix broth. Open an ampule of m-TGE or use an ampule breaker if necessary. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

For broth prepared from dehydrated medium, pipet approximately 2.0 mL of broth onto the pad using a sterile pipet. Drain excess medium from the petri dish and replace the lid.

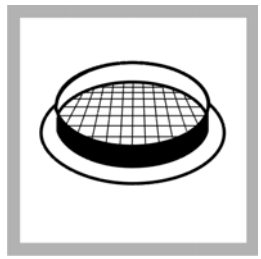
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter, grid side up, in the assembly.

Alternatively, a sterile, disposable filter unit may be used.



4. Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed. Filter the appropriate volume through the sterile 47 mm, 0.45µm, gridded membrane filter. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20–30 mL of sterile buffered dilution water.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ±0.5 °C for 24 hours.

8. Remove the dish from the incubator. Count colonies on membrane filters using a 10–15X microscope.

Bacterial colonies grown on m-TGE medium appear clear to cream in color.

Diluting the Sample

The pour plate method requires use of 1 mL, 0.1 mL, and 0.01 mL or 0.001 mL of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 mL, require the use of sample dilutions. These dilutions are prepared by pipetting 1 mL of undiluted sample into 99 mL of buffered dilution water. Diluting the sample allows 1 mL of diluted sample to be used instead of 0.01 mL of undiluted sample, and 0.1 mL of diluted sample instead of 0.001 mL of undiluted sample.

Selecting Sample Volumes/Dilutions

Select the sample volumes or dilutions to be used so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL of undiluted sample, 0.1 mL of undiluted sample and 1 mL of diluted sample (which equals 0.01 mL of undiluted sample). In examining sewage or turbid water, do not measure a 0.1-mL inoculum of the original undiluted sample, but do prepare an appropriate dilution.

Counting, Computing and Reporting Results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time, and the medium.

For example: 98 CFU/L, mL, 35 °C, 24 hours, m-TGE broth.

1 to 2, or fewer colonies per square — Count all of the colonies on the filter, and divide the results by the volume of original sample used.
For example, if there are 122 colonies on the filter, and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{\text{122 colonies}}{\text{10 mL sample}} = 12.2 \text{ CFU/mL}$$

3 to 10 colonies per square — Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.
For example, if you calculated an average of 8 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{8 colonies/square x 100}}{\text{0.1 mL sample}} = 8000 \text{ CFU/mL}$$

10 to 20 colonies per square — Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.

For example: if there are an average of 17 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{17 colonies/square x 100}}{\text{0.1 mL sample}} = 17,000 \text{ CFU/mL}$$

More than 20 colonies per square — If there are more than 20 colonies per square, record the count as > 2000 divided by the volume of original sample used.
For example, if the original volume of sample used were 0.01 mL, results would be > 2000/0.01 or > 200,000 CFU/mL.

Note: *Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.*

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Dilution Water, Buffered, sterile, 99-mL	25/pkg	1430598
m-TGE PourRite™ Ampules, glass, 2-mL	20/pkg	2373820

Required apparatus

Description	Unit	Catalog number
Absorbent Pads with dispenser, sterile, Gelman	1000/pkg	1491800
Ampule Breaker, PourRite™	each	2484600
Whirl-Pak Bags with declorinating agent, sterile, 180-mL	100/pkg	2075333
Filter Holder, magnetic coupling	each	1352900
Filtering Flask, 500-mL	each	54649
Forceps	each	2141100
Incubator, Culture, low profile, 110 VAC	each	2619200
Incubator, Culture, low profile, 220 VAC	each	2619202
Membrane filters, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Membrane filters, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Microscope, Compound	each	2942500
Petri Dish, polystyrene, sterile, disposable, without pad	100/pkg	1485299
Petri Dish, polystyrene, sterile, disposable, w/pad, Gelman	100/pkg	1471799
Petri Dish, polystyrene, sterile, disposable, w/pad, Millipore	150/pkg	2936300
Pump, vacuum, 110/115 VAC	each	2824800
Pump, vacuum, 220/230 VAC, Continental European Plug	each	2824802
Rubber Stopper, one hole, No. 8	6/pkg	211908
Rubber Tubing, 3.6-m	each	56019
Pipets, Serological, 10–11 mL, sterile, disposable	25/pkg	209798

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	each	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Counter, hand tally	each	1469600
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Isopropyl alcohol	500 mL	1445949
Microscope, Stereo Binocular	each	2942600
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Wicks, replacement, for alcohol burner 2087742	—	2097810

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Edition 7

Heterotrophic Bacteria

DOC316.53.01228

Pour Plate Method **Method 8242**

m-TSB/USP

Scope and Application: For water and wastewater.

Test preparation

Introduction

The Pour Plate Method, also known as the standard plate count, is simple to perform and is commonly used to determine heterotrophic bacteria density. This method does, however, have disadvantages that limit recovery of the maximum number of organisms. Tempered medium at 44–46 °C (111–115 °F) may cause heat shock to stressed bacteria and the nutritionally rich medium may decrease recovery of starved bacteria.

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultatively anaerobic heterotrophic bacteria in water. Bacteria occur singly or in pairs, chains, clusters or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. However, the heterotrophic plate count is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of source water.

Before starting the test:

See the *Introduction to Bacteria* for more information about preparing sample containers and collecting and preserving samples.
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.
Limit the number of samples to be plated at any one time so that no more than 20 minutes (preferably 10 minutes) elapse between the dilution of the first sample and the pouring of the last plate.
To save time, start the incubator before preparing the other materials. Set the incubator for the temperature required in the procedure (usually 35 ± 0.5 °C).
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Mark each pour plate, membrane filtration petri dish, or other sample container with the sample number, dilution, date, and any other necessary information. Take care not to contaminate the inside of the sample container in any way.

Pour plate procedure for heterotrophic bacteria m-TSB/USP, method 8242

1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish. **Do not touch the pad or the inside of the petri dish.**

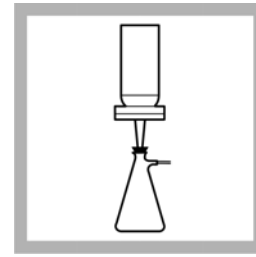
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

2. Invert ampules two or three times to mix broth. Open an ampule of m-TSB/USP or use an ampule breaker if necessary. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

For broth prepared from dehydrated medium, pipet approximately 2.0 mL of broth onto the pad using a sterile pipet. Drain excess medium from the petri dish and replace the lid.

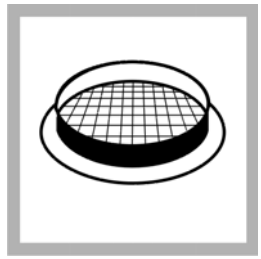
3. Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter, grid side up, in the assembly.

Alternatively, a sterile, disposable filter unit may be used.



4. Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed. Filter the appropriate volume through the sterile 47 mm, 0.45µm, gridded membrane filter. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20–30 mL of sterile buffered dilution water.

5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ± 0.5 °C for 24 hours.

8. Remove the dish from the incubator. Count colonies on membrane filters using a 10–15X microscope.

Bacterial colonies grown on m-TSB/USP medium appear clear to cream in color.

Diluting the Sample

The pour plate method requires use of 1 mL, 0.1 mL, and 0.01 mL or 0.001 mL of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 mL, require the use of sample dilutions. These dilutions are prepared by pipetting 1 mL of undiluted sample into 99 mL of buffered dilution water. Diluting the sample allows 1 mL of diluted sample to be used instead of 0.01 mL of undiluted sample, and 0.1 mL of diluted sample instead of 0.001 mL of undiluted sample.

Selecting Sample Volumes/Dilutions

Select the sample volumes or dilutions to be used so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL of undiluted sample, 0.1 mL of undiluted sample and 1 mL of diluted sample (which equals 0.01 mL of undiluted sample). In examining sewage or turbid water, do not measure a 0.1-mL inoculum of the original undiluted sample, but do prepare an appropriate dilution.

Counting, Computing and Reporting Results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time, and the medium.

For example: 98 CFU/L, mL, 35 °C, 24 hours, m-TSB/USP broth.

1 to 2, or fewer colonies per square — Count all of the colonies on the filter, and divide the results by the volume of original sample used.
For example, if there are 122 colonies on the filter, and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{122 \text{ colonies}}{10 \text{ mL sample}} = 12.2 \text{ CFU/mL}$$

3 to 10 colonies per square — Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.
For example, if you calculated an average of 8 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{8 \text{ colonies/square} \times 100}{0.1 \text{ mL sample}} = 8000 \text{ CFU/mL}$$

10 to 20 colonies per square — Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.

For example: if there are an average of 17 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{17 \text{ colonies/square} \times 100}{0.1 \text{ mL sample}} = 17,000 \text{ CFU/mL}$$

More than 20 colonies per square — If there are more than 20 colonies per square, record the count as > 2000 divided by the volume of original sample used.
For example, if the original volume of sample used were 0.01 mL, results would be > 2000/0.01 or > 200,000 CFU/mL.

Note: *Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.*

Consumables and replacement items

Required media and reagents

Description	óUnit	Catalog number
Dilution Water, Buffered, sterile, 99-mL	25/pkg	1430598
m-TSB/USP Broth Ampules, plastic, 2-mL	20/pkg	2812650

Required apparatus

Description	Unit	Catalog number
Absorbent Pads with dispenser, sterile, Gelman	1000/pkg	1491800
Ampule Breaker, PourRite™	each	2484600
Whirl-Pak Bags with declorinating agent, sterile, 180-mL	100/pkg	2075333
Filter Holder, magnetic coupling	each	1352900
Filtering Flask, 500-mL	each	54649
Forceps	each	2141100
Incubator, Culture, low profile, 110 VAC	each	2619200
Incubator, Culture, low profile, 220 VAC	each	2619202
Membrane filters, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Membrane filters, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Microscope, Compound	each	2942500
Petri Dish, polystyrene, sterile, disposable, without pad	100/pkg	1485299
Petri Dish, polystyrene, sterile, disposable, w/pad, Gelman	100/pkg	1471799
Petri Dish, polystyrene, sterile, disposable, w/pad, Millipore	150/pkg	2936300
Pump, vacuum, 110/115 VAC	each	2824800
Pump, vacuum, 220/230 VAC, Continental European Plug	each	2824802
Rubber Stopper, one hole, No. 8	6/pkg	211908
Rubber Tubing, 3.6-m	each	56019
Pipets, Serological, 10–11 mL, sterile, disposable	25/pkg	209798

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	each	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Counter, hand tally	each	1469600
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Isopropyl alcohol	500 mL	1445949
Microscope, Stereo Binocular	each	2942600
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Wicks, replacement, for alcohol burner 2087742	—	2097810

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Edition 7

Heterotrophic Bacteria

DOC316.53.01229

Pour Plate Method **Method 8241**

Plate Count Agar[1]

Scope and Application: For water and wastewater

[1] This method meets or exceeds the specification criteria stated in *Standard Methods for the Examination of Water and Wastewater*, 19th edition, Method 9215 B. Pour Plate Method.

Test preparation

Before starting the test:

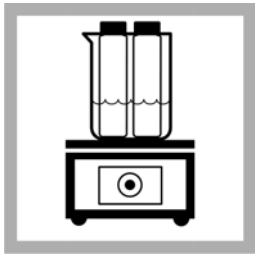
See the *Introduction to Bacteria* for more information about preparing sample containers and collecting and preserving samples.
Limit the number of samples to be plated at any one time so that no more than 20 minutes (preferably 10 minutes) elapse between the dilution of the first sample and the pouring of the last plate.
To save time, start the incubator before preparing the other materials. Set the incubator for the temperature required in the procedure (usually 35 ± 0.5 °C).
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
Mark each pour plate, membrane filtration petri dish, or other sample container with the sample number, dilution, date, and any other necessary information. Take care not to contaminate the inside of the sample container in any way.

Introduction

The Pour Plate Method, also known as the standard plate count, is simple to perform and is commonly used to determine heterotrophic bacteria density. This method does, however, have disadvantages that limit recovery of the maximum number of organisms. Tempered medium at 44–46 °C (111–115 °F) may cause heat shock to stressed bacteria and the nutritionally rich medium may decrease recovery of starved bacteria.

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultatively anaerobic heterotrophic bacteria in water. Bacteria occur singly or in pairs, chains, clusters or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. However, the heterotrophic plate count is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of source water.

Pour plate procedure for heterotrophic bacteria, method 8241



1. Melt the sterile solid agar medium by placing a tube of Plate Count Agar in a beaker of boiling water. (Each tube contains enough medium for two plates.)

Avoid prolonged exposure to unnecessarily high temperatures during and after melting.

When the medium is melted in two or more batches, use all of each batch in order of melting, provided the contents remain fully melted. Discard any melted agar that contains precipitate.

Loosen caps before heating to make it easier to pour just after the media has melted.

2. Keep the melted medium in a water bath, between 44–46 °C, until used.

Do not depend on the sense of touch to indicate the proper temperature when pouring the agar.

Place a thermometer in the water bath to ensure correct temperature range—to avoid contamination, the thermometer should be in a separate container of melted agar or water, not in the agar tubes to be plated.

3. Pipet the appropriate amount of undiluted or diluted sample (1 mL or 0.1 mL—see *Diluting the Sample*) into the sterile petri dish. Prepare at least two plates for each different volume of undiluted or diluted sample used.

Thoroughly mix all undiluted and diluted samples by inverting about 25 complete up-and- down (or back-and-forth) movements. Or, use a mechanical shaker to shake samples or dilutions for 15 seconds.

Lift the lid of the petri dish just high enough to insert the pipet. Hold the pipet at a 45 ° angle, with the tip just touching the bottom of the dish. Allow enough time (at least 2–4 seconds) for the pipet to drain.

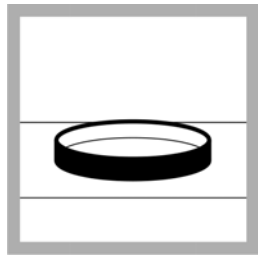
4. Pour at least 10 to 12 mL of liquefied medium (½ of the contents of Plate Count Agar tube) into the dish by gently lifting the cover just high enough to pour.

Avoid spilling the medium on the outside of the container or on the inside of the dish lid when pouring. Replace the lid when finished.

Pour plate procedure for heterotrophic bacteria, method 8241 (continued)

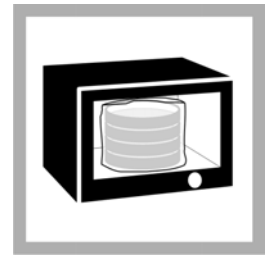
5. Mix the melted medium thoroughly with the sample in the petri dish by swirling in a figure-eight motion with the petri dish on the bench top.

Do not invert the petri dish to mix.



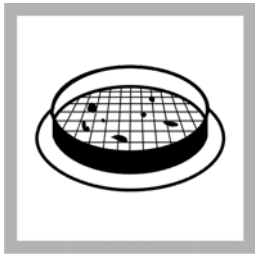
6. Place the plates on a level surface and let them solidify. This generally takes 10 minutes.

7. Invert the plates, place them in a plastic bag, and seal the bag. Place the bag in an incubator that has been prewarmed to 35 °C.



8. Incubate the plates for 48 ± 3 hours at 35 ± 0.5 °C.

During incubation, maintain humidity within the incubator so that plates will not have moisture weight loss greater than 15%. A pan of water placed at the bottom of the incubator may be sufficient. For incubation in non-humidified incubators, make certain that the plastic bags are tightly sealed.



9. Using a Quebec Colony Counter, count all colonies on the plates promptly after incubation. See *Interpreting and Reporting Results*.

Diluting the Sample

The pour plate method requires use of 1 mL, 0.1 mL, and 0.01 mL or 0.001 mL of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 mL, require the use of sample dilutions. These dilutions are prepared by pipetting 1 mL of undiluted sample into 99 mL of buffered dilution water. Diluting the sample allows 1 mL of diluted sample to be used instead of 0.01 mL of undiluted sample, and 0.1 mL of diluted sample instead of 0.001 mL of undiluted sample.

Note: *Standard microbiological procedures require at least 2 tests per sample.*

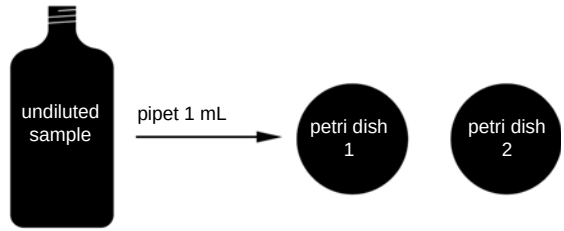


Figure 17 Dilution factor 1, 1-mL sample volume

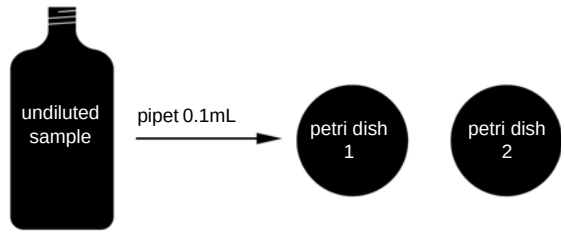


Figure 18 Dilution factor 10, 0.1-mL sample volume

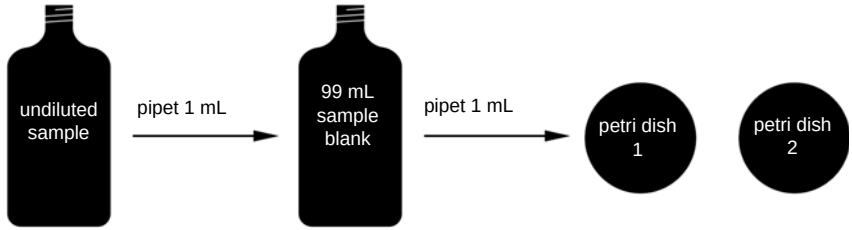


Figure 19 Dilution factor 100, 0.01-mL sample volume

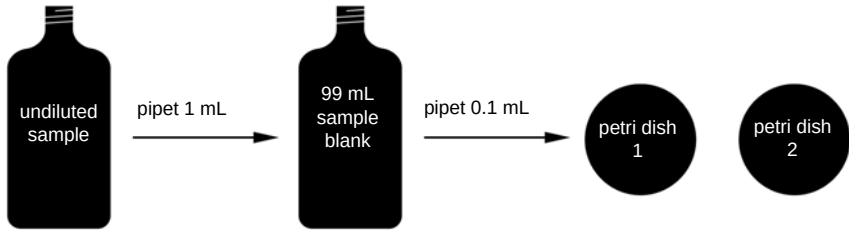


Figure 20 Dilution factor 1000, 0.001-mL sample volume

Selecting Sample Volumes/Dilutions

Select the sample volumes or dilutions to be used so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL of undiluted sample, 0.1 mL of undiluted sample and 1 mL of diluted sample (which equals 0.01 mL of undiluted sample). In examining sewage or turbid water, do not measure a 0.1-mL inoculum of the original undiluted sample, but do prepare an appropriate dilution.

Interpreting and Reporting Results

Count all colonies on selected plates promptly after incubation. If count must be delayed temporarily, store plates at 5–10 °C for no more than 24 hours, but avoid routine delays.

Quebec Colony Counters feature a built-in grid to simplify counting. The easiest way to count colonies is to follow a back and forth pattern, moving down the grid. See the *Colony counting technique* figure.

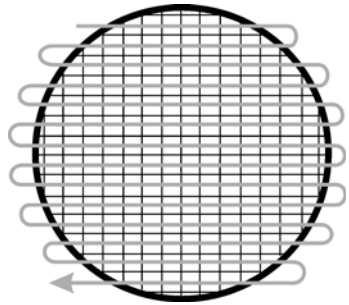


Figure 21 Colony counting technique

Report all counts as colony-forming units (CFU)/mL. Include in the report the method used, the incubation temperature and time, and the medium. ***For example,*** 75 CFU/mL, pour plate method, 35 °C/48 hours, plate count agar.

Generally, results are obtained by averaging the number of colonies on all plates from the same undiluted or diluted sample volume, and multiplying by a dilution (described below). In this case, results should be rounded to two significant digits to avoid creating false precision. For three-digit results, raise the middle digit if the last digit is 5 or greater. Retain the middle digit if the last digit is 4 or smaller. The last digit will be zero.
For example, 143 would become 140, 255 would become 260. Two digit numbers require no rounding.

Be familiar with the following terms before counting and reporting results:

Average number of colonies/plate —The average number of colonies per plate is derived by dividing the total number of colonies on all plates that were inoculated with the same sample volume or dilution volume, and dividing that sum by the number of plates used.
For example, if two plates were each inoculated with 1 mL of diluted sample, and there were 89 colonies on one plate and 103 on the other, then the average number of colonies/plate would be:

$$\frac{89 \text{ colonies} + 103 \text{ colonies}}{2 \text{ plates}} = 96 \text{ colonies}$$

Colony-forming units (CFU)/mL —This is the unit used for reporting bacterial density. To derive the number of CFU/mL, multiply the **average number of colonies/plate** by the **dilution factor** of the incubated sample.

> ***Note:*** *In some instances where a large number of colonies are observed, the average number of colonies/plate is obtained by adding colonies counted only in a specified number of squares on each plate.*

Dilution factor —The dilution factor is the reciprocal of the volume of original, undiluted sample plated, and is used to standardize the results according to the sample volume.
For example, if 1 mL of original sample was used, the dilution factor is 1.
If 0.1 mL of original sample was used, the dilution factor is 10. The dilution factor for 1 mL of diluted sample (0.01 mL of original sample) is 100, and the dilution factor for 0.1 mL of diluted sample (0.001 mL of original sample) is 1000.

Representative colony distribution—When counting colonies in a specified number of squares (as seen through the colony counter), count those squares that appear to have an average number of colonies. Avoid counting squares that have many less or many more colonies than most of the other squares on the plate.

Spreaders—Spreaders are colonies of bacteria which grow in such a way that they appear to be “spread” across the plate. See the *Spreader growth* figure.

Figure 22 Spreader growth

It is preferable when counting and recording results, to consider plates having between 30 and 300 colonies. However, this is not always the case, so when counting and recording colonies, choose the situation that best describes
your results.

If spreaders are encountered on the plates selected, count colonies on representative portions only when the colonies are well distributed in
spreader-free areas, and the area covered by the spreaders does not exceed
one-half of the plate area.

When spreading colonies must be counted, count each of the following types as one colony:

1. A chain of colonies that appears to be caused by disintegration of a bacterial clump as agar and sample were mixed.
2. A spreader that develops as a film of growth between the agar and the bottom of the petri dish.
3. A colony that forms in a film of water at the edge or over the agar surface.

Count as **individual** colonies the similar-appearing colonies growing in close proximity but not touching, provided that the distance between them is at least equal to the diameter of the smallest colony.

Also count as **individual** colonies those colonies which are touching, but are different in appearance, such as morphology or color.

To obtain results, multiply the **average number of colonies/plate** by the **dilution factor**. Report counts as CFU/mL.

If plates have excessive spreader growth, report as “spreaders” (Spr). When plates are uncountable because of missed dilution, accidental dropping, or contamination, or the control plates indicate that the medium or other material or labware was contaminated, report as “laboratory accident” (LA).

No colonies— If plates from all dilutions of any sample have no colonies, report the count as less than one (<1) times the dilution factor for the largest volume of original sample used.
For example, if no colonies develop using 0.1 mL of original sample, report the count as less than 10 (<10) estimated CFU/mL.

Less than 30 colonies/plate—Ordinarily, no more than 1.0 mL of sample is plated. Therefore, when the total number of colonies developing from 1.0 mL is less than 30, record the number of colonies as CFU/mL.

30 to 300 colonies/plate—Compute bacterial count per mL by multiplying the **average number** of colonies/plate by the **dilution factor**. Report counts as CFU/mL.

For example, 0.1 mL of undiluted sample was used to inoculate two plates. After incubation, the plates had colony counts of 115 and 145. The CFU/mL value is computed as follows:

$$\frac{115 \text{ colonies} + 145 \text{ colonies}}{2 \text{ plates}} \times 10 \text{ (dilution factor)} = 1300 \text{ CFU/mL}$$

Greater than 300 colonies/plate— If no plate has 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plates having a count closest to 300 colonies. Compute the count by multiplying the **average number of colonies/plate** by the **dilution factor**, and report as estimated CFU/mL.

Far more than 300 colonies/plate— If there are greater than 300 colonies/plate, *do not* report the result as "too numerous to count" (TNTC). Instead, follow guidelines for reporting if there are less than 10 colonies/cm^2:

Less than 10 colonies/cm^2 — If there are fewer than 10 colonies per cm^2 (one "square" as seen through the colony counter), then count colonies in 13 squares having **representative colony distribution**.

a. Select seven consecutive squares horizontally across the plate, and six consecutive squares vertically, being careful not to count a square more than once. See the *Colony counting, > 300 colonies* figure.

b. Add the number of colonies in each square.

c. Multiply this sum by 4.38 when the plate area is 57 cm^2 (disposable plastic plates).

d. Multiply the sum by 5 when the plate area is 65 cm^2 (glass plates). To determine colony-forming units (CFU)/mL, compute the **average number of colonies/plate** and multiply the result by the dilution factor. Report as estimated CFU/mL.

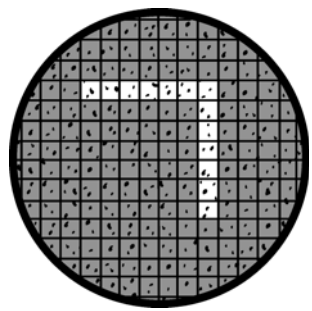


Figure 23 Colony counting, > 300 colonies

Note: *One thousand is the dilution factor for 0.1 mL of diluted sample. This is the sample volume that should be used when bacterial counts are this high.*

More than 10 colonies/cm^2—When there are more than 10 colonies per cm^2 (one "square" as seen through the colony counter), count colonies in four squares having **representative colony distribution**. Add the number of colonies in these four squares, and divide the sum by 4, to get the average number of
colonies/square. Multiply this number by 57 when the plate area is 57 cm^2 (disposable plastic plates). Multiply this number by 65 when the plate area is 65 cm^2 (glass plates). To determine CFU/mL, compute the **average number of colonies/plate** and multiply the result by 1000 (see "Note" below). Report as estimated CFU/mL.

Avoiding Errors
Avoid inaccuracies in counting due to damaged or dirty optics that impair vision, or due to failure to recognize colonies. Be careful not to contaminate plates due to improper handling. Laboratory workers who cannot duplicate their own counts on the same plate within 5%, and counts of other analysts within 10%, should discover the cause and correct such disagreements.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dilution Water, Buffered, sterile, 99-mL	25/pkg	1430598
Plate Count Agar Tubes	20/pkg	2406720

Required apparatus

Description	Unit	Catalog number
Bags, Whirl-Pak®[1] with dechlorinating agent, sterile, 180-mL	100/pkg	2075333
Bags, Whirl-Pak without dechlorinating agent, sterile, 205-mL	100/pkg	2233199
Bags, Whirl-Pak without dechlorinating agent, sterile, 710-mL	10/pkg	1437297
Beaker, 250-mL	each	50046
Bottle, sample, glass, with cap, 118-mL	3/pkg	2163103
Clamp, Test Tube	each	63400
Colony Counter, Quebec, 110 VAC, 60 Hz	each	2252100
Colony Counter, Quebec, 220 VAC, 50 Hz	each	2252102
Counter, hand tally	each	1469600
Dish, Petri, 100 x 15 mm, sterile, disposable	20/pkg	2178901
Dish, Petri, 100 x 15 mm, sterile, disposable	500/pkg	2178900
Germicidal Cloths	50/pkg	2463200
Hot Plate, 120 VAC, 50/60 Hz	each	2881500
Hot Plate, 240 VAC, 50/60 Hz	each	2881502
Incubator, Culture, Low-Profile, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, Low-Profile, 220 VAC, 50/60 Hz	each	2619202
Pipets, Serological, 1 mL, sterile, disposable	50/pkg	2092835
Pipets, Serological, 10–11 mL, sterile, disposable	25/pkg	209798
Thermometer -20 to 110 °C, non-mercury	each	2635702

[1] Whirl-Pak is a registered trademark of Nasco, Inc.

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Edition 7

Total Aerobic Bacteria, Yeasts and Molds

DOC316.53.01223

Total Aerobic Bacteria (Amber)/Yeast and Mold (Red)
Total Aerobic Bacteria (Amber)/Total Coliform (Red)
Total Aerobic Bacteria (Amber)/Disinfection Control (Purple)

Paddle Testers

Scope and Application: For detection of contamination of equipment that contacts food, cosmetics or pharmaceuticals, boiler and cooling tower water, laboratory surfaces requiring aseptic conditions and kitchen surfaces in restaurants and hotels

Test preparation

Before starting the test:

To ensure accurate results, read carefully before proceeding.
Paddle testers have a 12-month shelf life from date of manufacture.
Paddle Testers are most suitable for semi-quantitative screening. Monitoring with Paddle Testers over time will provide a good indication of how the microbial load is changing at a given site.
Wash hands thoroughly with soap and water.
For greater accuracy when interpreting results, count the colonies with the aid of the molded-in grid.

Paddle testers in liquid

1. Remove the paddle from the vial. Do not touch the media.

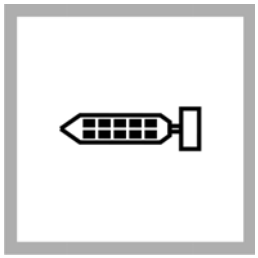
2. Dip the paddle into a liquid sample. Immerse all the growth media on the paddle into the liquid.

3. Remove the paddle from the liquid and allow excess liquid to drip off. Do not shake. Return the paddle to the vial and screw on the cap.

4. Incubate the Paddle Tester.

See the *Incubation of Paddle Testers* for incubation times.

Paddle testers in liquid (continued)

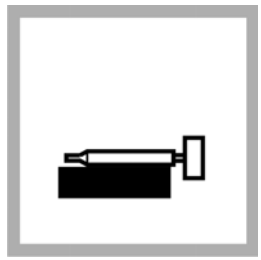


5. Refer to the *Bacterial colony density* and *Yeast and mold colony density* tables for results.

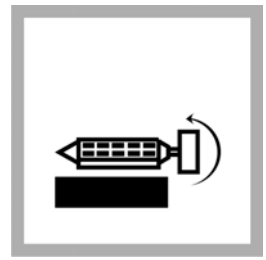
Sterilize the paddles before disposal. (See *Disposing of Paddles* table.)

Paddle testers on solid and flat surfaces

1. Remove the paddle from the vial. Do not touch the media.



2. Press one side of the paddle to the surface to be tested.

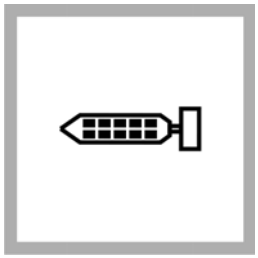


3. Turn the paddle over and press the other side to a new area of the test surface. Return the paddle to the vial and screw on the cap.

4. Incubate the Paddle Tester.

See the *Incubation of Paddle Testers* table for incubation times.

Paddle testers on solid and flat surfaces (continued)



5. Refer to the *Bacterial colony density* and *Yeast and mold colony density* tables for results.

Sterilize the paddles before disposal. (See *Disposing of Paddles*.)

Table 390 Incubation of Paddle Testers

Incubation of Paddle Testers	Incubation Temperature	Examine at:
Yeast and Mold	25 to 30 °C	Bacteria: 24 to 48 hours Yeast and Mold: 48 hours and up to 120 hours (5 days)
Total Coliform	35 to 37 °C	24 to 48 hours
Disinfection Control	35 to 37 °C	24 to 48 hours

Disposing of Paddles

To sterilize Paddle Testers before disposal:

- Autoclave for 15 minutes at 121 °C (250 °F) at 103 kPa (15 psi) or
- Pour 10 mL of household bleach (5.25% NaOCl) into the vial and allow it to sit for a minimum of 30 minutes.

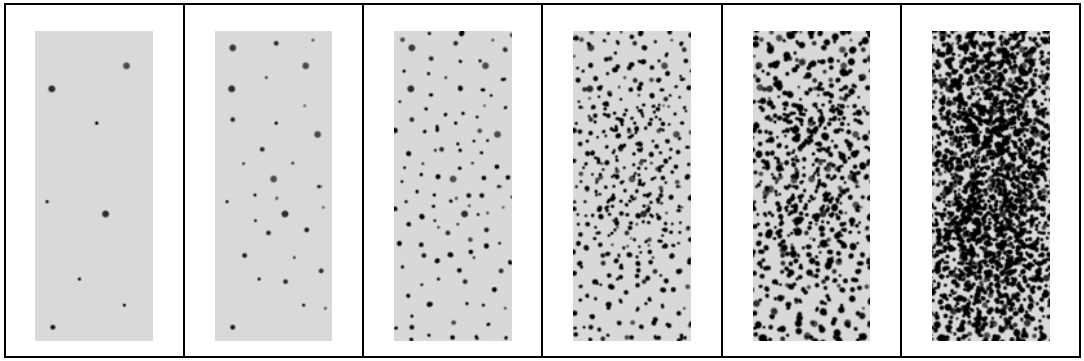
Interpreting the level of Contamination for total bacteria or total coliform bacteria

Identify the slide that most closely matches the sample. Use the density value above the slide.

Table 391 Bacterial colony density

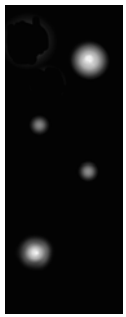
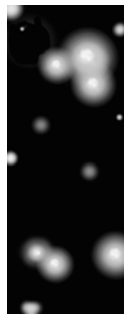
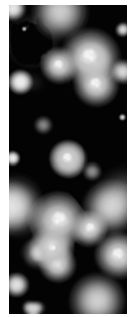
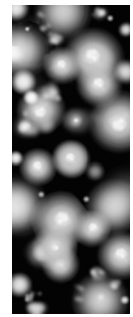
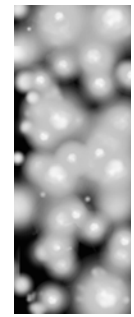
100 (10^2)	1000 (10^3)	10,000 (10^4)	100,000 (10^5)	1,000,000 (10^6)	10,000,000 (10^7)

Table 391 Bacterial colony density



Interpreting the level of contamination for yeast and mold

Table 392 Yeast and mold colony density

10 (10^1)	100 (10^2)	1000 (10^3)	10,000 (10^4)	100,000 (10^5)	
					

Consumables and replacement items

Required reagents and media

Description	Unit	Catalog number
Total Aerobic Bacteria/Yeast and Mold Paddle Testers	10/pkg	2610810
Total Aerobic Bacteria/Total Coliform Paddle Testers	10/pkg	2610910
Total Aerobic Bacteria/Disinfection Control Paddle Testers	10/pkg	2619510

Required apparatus

Description	Unit	Catalog number
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Beaker, 100-mL	each	50042H
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199

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Edition 7

Electrochemistry

Conductivity

DOC316.53.01199

USEPA[1] Direct Measurement Method[2] **Method 8160**

(0.01 µS/cm to 200.00 mS/cm) **Conductivity Meter**

Scope and Application: For water and wastewater

[1] USEPA accepted for reporting for Standard method 2510-B

[2] Procedure is equivalent to Standard Method 2510-B for wastewater.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 393 Instrument-specific information

Meter	Standard probe	Rugged probe[1]
HQ40d	CDC40101 CDC40103	CDC40105 CDC40110 CDC40115 CDC40130
HQ30d	CDC40101 CDC40103	CDC40105 CDC40110 CDC40115 CDC40130
HQ14d	CDC40101 CDC40103	CDC40105 CDC40110 CDC40115 CDC40130
*sens**ion***™ 5	5197500 5197503	—
*sens**ion***™ 7	5197500 5197503	—

[1] Designed for field use.

Before starting the test:

Collect samples in clean plastic or glass bottles.
Analyze samples as soon as possible after collection. However, samples may be stored at least 24 hours by cooling to 4 °C (39 °F) or below (all storage temperatures have changed to 0 to 6 °C as per the EPA MUR, March 2007). When measuring solutions that are not at the reference temperature, the meter automatically adjusts the conductivity value to the reference temperature from 20 or 25 °C.
Water samples containing oils, grease, or fats will coat the electrode and affect the accuracy of the readings. If this occurs, clean the probe with a strong detergent solution, then thoroughly rinse with deionized water.
Mineral build-up on the probe can be removed with a diluted 1:1 Hydrochloric Acid Solution. Refer to the meter user manual.
Calibration instructions are given in the operation section of the meter manual. For most accurate results calibrate before use, or check the accuracy of the meter with a known conductivity standard.
Measurement errors can occur if the appropriate temperature correction value is not chosen. Refer to the *Temperature correction* table for typical correction values.

Collect the following items:

Description	Quantity
One of the following meter/sensor combinations:	1
HQd meter and conductivity IntelliCAL™ probe	
*sens**ion*** meter and conductivity electrode	1
One of the following Hach standards:	
NaCl Standard Solution, 180 ± 10 µS/cm	1
NaCl Standard Solution, 1000 ± 10 µS/cm	1
NaCl Standard Solution, 1990 ± 10 µS/cm	1
NaCl Standard Solution, 18 ± 10 mS/cm	1
Radiometer Analytical Certified Conductivity Standards:	
KCl, 1 Demal, 111.3 mS/cm ± 0.5% at 25 °C	500 mL
KCl, 0.1 Demal, 12.85 mS/cm ± 0.35% at 25 °C	500 mL
KCl, 0.01 Demal, 1408 S/cm ± 0.5% at 25 °C	500 mL
NaCl, 0.05%, 1015 S/cm ± 0.5% at 25 °C	500 mL
KCl Conductivity Standards:	
0.1 Molar KCl, 12.88 mS/cm at 25 °C	500 mL
0.01 Molar KCl, 1413 S/cm at 25 °C	500 mL
0.001 Molar KCl, 148 S/cm at 25 °C	500 mL
Beaker, poly, 100-mL	1

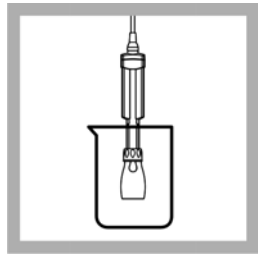
See *Consumables and replacement items* for reorder information.

Conductivity

1. Refer to the operation section of the meter manual to prepare the conductivity electrode and meter.

The meter will select the range automatically.

For most accurate results, calibrate the meter before use or check the accuracy of the meter with a known conductivity standard. Refer to the meter user manual for calibration and measurement options.



2. Laboratory tests: Immerse the probe in a beaker containing the sample solution. Move the probe up and down and tap it on the beaker to remove bubbles from the electrode.

Field tests: Immerse the probe in the sample solution. Move the probe up and down to remove bubbles from the electrode.

The vent holes should be completely submerged.

3. Turn the meter on. Make sure that the meter is set to measure conductivity.

4. Rinse the probe thoroughly with deionized water after each measurement.

To display other units such as TDS, salinity or resistivity (HQd only), refer to the meter user manual.

Conversions

The *Unit conversion* table provides equations for converting the conductivity readings to other units of measure.

Table 394 Unit conversion

From	To	Use this Equation
mS/cm	µS/cm	mS/cm x 1000
µS/cm	mS/cm	µS/cm x 0.001
µS/cm	µmhos/cm	µS/cm x 1
mS/cm	mmhos/cm	mS/cm x 1
µS/cm	mg/L TDS	µS/cm x 0.5[1]
g/L TDS	mg/L TDS	g/L TDS x 1000
mS/cm	g/L TDS	mS/cm x 0.5
mg/L TDS	g/L TDS	mg/L TDS x 0.001
mg/L TDS	gpg TDS	mg/L TDS x 0.05842
g/L TDS	gpg TDS	g/L TDS x 58.42
µS/cm	ohms cm	1,000,000 ÷ µS/cm
mS/cm	ohms cm	1,000 ÷ mS/cm

[1] TDS is an empirically-derived value from the conductivity measurement. A value of 0.5 is selected here for simplicity and suitability to a wide variety of waters. The *sens**ion*** 5 uses a more complex algorithm, based on additional factors, such as temperature, to determine TDS.

The *Temperature correction* table shows typical temperature correction values for selected solutions using the linear temperature correction option.

Table 395 Temperature correction

Solution	Percent per °C
Ultrapure Water	4.55
Salt (NaCl)	2.125
NaOH	1.72
Dilute Ammonia	1.8810
10% HCl	1.325
5% Sulfuric Acid	0.9698

Interferences

When measuring conductivity, the following items should be considered in order to ensure accurate results:

- If measuring very low levels of conductivity, protect the sample from atmospheric gases (carbon dioxide, ammonia). These gases dissolve readily in water and may cause a rapid change in conductivity. To minimize these effects, boil the sample, then place in a covered container, such as a Low Ionic Strength (LIS) chamber for cooling.
- To remove the conductivity with hydroxide ions, neutralize by adding 4 drops of Phenolphthalein Indicator Solution to 50 mL of sample, then adding Gallic Acid Solution, drop-wise, until the pink color completely disappears.

Accuracy check

Pour a Sodium Chloride Standard Solution (with a conductivity value in the same range as the sample) into a beaker. Perform the conductivity measurements as described above. The conductivity reading should be the same (within accuracy limits) as listed on the Standard Solution label if the meter is calibrated correctly. Calibration can be performed using this solution. Refer to the meter user manual.

Method performance

The accuracy of a conductivity measurement is dependent on many factors associated with the overall system, including the meter, meter settings, choice of electrode and conductivity standards being used during calibration. Refer to the appropriate electrode, meter manual and standard certificate of analysis to help determine system performance.

Summary of method

Electrolytic conductivity is the capacity of ions in a solution to carry electrical current and is the reciprocal of the solution resistivity. Current is carried by inorganic dissolved solids (e.g., chloride, nitrate, sulfate, and phosphate anions) and cations (e.g., sodium, calcium, magnesium, iron, and aluminum). Organic material like oils, phenols, alcohols, and sugars do not carry electrical current well and thus do not have enough conductivity for a useful estimate of concentration.

Measuring conductivity is done by measuring the resistance occurring in an area of the test solution defined by the probe's physical design. Voltage is applied between the two electrodes immersed in the solution, and the voltage drop caused by the resistance of the solution is used to calculate conductivity per centimeter. The basic unit of measure for conductivity is the Siemen (or mho), the reciprocal of the ohm in the resistance measurement. Because ranges normally found in aqueous solutions are small, milliSiemens/cm (10^{-3} S or S/cm) and microSiemens/cm (10^{-6} S or µS/cm) are most commonly used.

Consumables and replacement items

Required apparatus

Description	Quantity	Unit	Catalog number
Select one meter and probe combination:			
HQ40d Meter	1	each	HQ40d53000000
HQ30d Meter	1	each	HQ30d53000000
HQ14d Meter	1	each	HQ14d53000000
IntelliCAL Conductivity Probe, standard, with 1m cable	1	each	CDC40101
IntelliCAL Conductivity Probe, standard, with 3m cable	1	each	CDC40103
IntelliCAL Conductivity Probe, rugged, with 5m cable	1	each	CDC40105
IntelliCAL Conductivity Probe, rugged, with 10m cable	1	each	CDC40110
IntelliCAL Conductivity Probe, rugged, with 15m cable	1	each	CDC40115
IntelliCAL Conductivity Probe, rugged, with 30 m cable	1	each	CDC40130
sens*ion* meters and probes. Select one meter and probe combination:			
*sens**ion*** 5	1	each	5180000
*sens**ion*** 7	1	each	5450000
Conductivity probe, with 1 m cable	1	each	5197500
Conductivity probe, with 3 m cable	1	each	5197503

Recommended standards

Description	Unit	Catalog number
Hach, NaCl Conductivity Standards:		
Sodium Chloride Standard Solution, 180 ±10 mS/cm, 90 ±1 mg/L TDS	100 mL	2307542
Sodium Chloride Standard Solution, 1000 ±10 mS/cm, 500 ±5 mg/L TDS	100 mL	1440042
Sodium Chloride Standard Solution, 1990 ±20 mS/cm, 995 ±10 mg/L TDS	100 mL	210542
Sodium Chloride Standard Solution, 18,000 ±50 mS/cm, 9000 ±25 mg/L TDS	100 mL	2307442
Radiometer Analytical, Certified Conductivity Standards:		
KCl, 1 Demal, 111.3 mS/cm ± 0.5% at 25 °C	500 mL	S51M001
KCl, 0.1 Demal, 12.85 mS/cm ± 0.35% at 25 °C	500 mL	S51M002
KCl, 0.01 Demal, 1408 µS/cm ± 0.5% at 25 °C	500 mL	S51M003
NaCl, 0.05%, 1015 µS/cm ± 0.5% at 25 °C	500 mL	S51M004
KCl Conductivity Standards:		
0.1 Molar KCl, 12.88 mS/cm at 25 °C	500 mL	C20C250
0.01 Molar KCl, 1413 µS/cm at 25 °C	500 mL	C20C270
0.001 Molar KCl, 148 µS/cm at 25 °C	500 mL	C20C280

Optional reagents and apparatus

Description	Unit	Catalog number
Beaker, poly, 100-mL	each	108042
Gallic Acid Solution	50 mL SCDB	1442326
Hydrochloric Acid Solution, 1:1	500 mL	88449
Low Ionic Strength Chamber (LIS)	each	5189900
Phenolphthalein Indicator Solution	15 mL SCDB	16236
Wash Bottle, 125-mL	each	62014
Water, deionized	4 L	27256

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Edition 7

Fluoride in Acid Solutions

DOC316.53.01236

Direct Measurement ISE Method **Method 8323**

0.1 to 100.0 mg/L F **ISE Electrode**

Scope and Application: For industrial waters (solutions below a pH of 5)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 396 Instrument-specific information

Meter	Electrode
*sens**ion***™ 4 meters	5192800
*sens**ion***™ 2 meters[1]	5192800

[1] The user must construct the calibration curve with the *sens**ion*** 2 meter.

Before starting the test:

Refer to the meter user manual for meter operation. Refer to electrode manual for electrode maintenance and care.
Prepare the electrode. Refer to *Electrode assembly* and *Condition the electrode* in this procedure.
In solutions below a pH of 5, hydrogen ion complexes some of the fluoride ions, forming HF or HF_2^-, which the electrode will not detect. To free the complexed fluoride, adjust the pH of the solution so it is weakly acidic to weakly basic before analysis.
Do not use a strong base (such as sodium hydroxide) for adjustment since the amount of base added will vary from sample to sample. Using a strong base can also change the ionic strength of the sample and alter measurement accuracy.
Dilution of samples and standards with a large excess of sodium acetate will buffer the pH and help adjust the total ionic strength of samples and standards to the same level.

Collect the following items:

Description	Quantity
Fluoride ISA buffer pillows (TISAB)	1 mL
Sodium acetate, ACS	varies
Fluoride standard solutions:	
10.00-mg/L F or	varies
2.00-mg/L F or	varies
100.0-mg/L F	varies
Potassium Chloride Reference Electrolyte Gel Cartridges	1
Water, deionized	varies

Collect the following items: (continued)

Description	Quantity
Beaker, 50-mL, polypropylene	3
Tensette pipet, 1.0–10.0 mL or	1
25 mL Class A volumetric pipet	1
Class A 1000 mL volumetric	1
Bottle, wash, 500-mL	1
Cylinder, graduated, 25-mL, poly	3
Platinum Series Fluoride Combination Electrode, BNC	1
*sens**ion***™2 Portable pH/ISE Meter.	1
OR	
*sens**ion***™4 Laboratory pH/ISE Meter	1
Stir Bar, 7/8 X 3/16 in. (22.2 x 4.8 cm)	4
Stirrer, electromagnetic with stand and stir bar	1

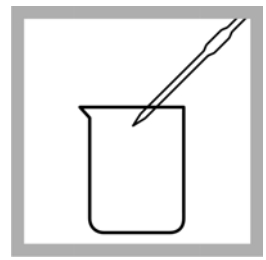
See *Consumables and replacement items* for reorder information.

Fluoride in Acid Solutions method

1. Prepare a 15% sodium acetate solution by dissolving 150 g of reagent grade sodium acetate in 1000 mL of deionized water. Prepare enough solution to dilute all the samples and standards.

2. Using deionized water, prepare a background solution containing all the sample matrix components except fluoride. Use this solution to prepare the standards.

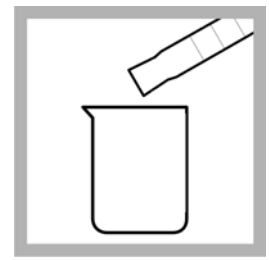
If a standard prepared with background solution gives the same reading (after sodium acetate dilution) as standard prepared with pure sodium acetate, it is not necessary to prepare a background solution.



3. For calibration, prepare fluoride standards by adding fluoride to the background solution.

Prepare fresh standards every two weeks if standards are less than 10 mg/L F^-.

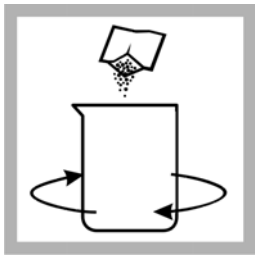
When testing with a direct reading fluoride meter, use two standards. When testing with a pH/mV meter, use three standards.



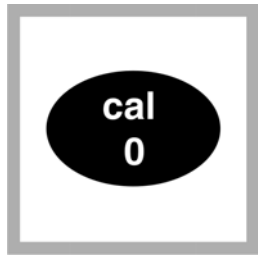
4. Dilute each standard 10:1 with sodium acetate solution (9 parts sodium acetate solution to 1 part standard) to prepare the buffered solution. Mix thoroughly.

For example, measure 3 mL of standard (with background solution) and dilute it with 27 mL of sodium acetate solution.

Fluoride in Acid Solutions method (continued)



5. Add the contents of one Fluoride Total Ionic Strength Adjustment Buffer (TISAB) Powder Pillow to 25 mL of the prepared buffered sample. Stir to dissolve.



6. Turn the meter on. Set the electrode type to BNC. Set the units to mg/L. Press **CAL**.

Refer to the meter user manual for details.

7. Place the beaker with the 1-mg/L prepared buffered standard on a magnetic stirrer. Stir at a moderate rate.

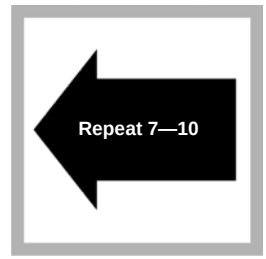
8. Place the electrode into the standard.

9. Edit the display to show the concentration of the standard.

Press **ENTER** and accept the concentration.

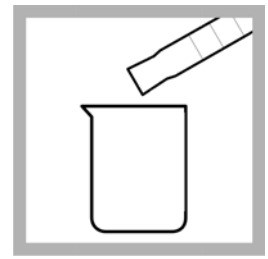
10. The display will show **Stabilizing** until the measurement is complete.

Remove the electrode from the standard solution Rinse with deionized water and blot dry.



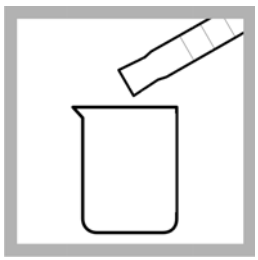
11. Repeat steps 7–10 for the 10-mg/L and 100 mg/L standards.

After the last standard is measured, store the calibration in the meter. Refer to the meter user manual for details.



12. Measure the sample into 50-mL beakers to measure 25 mL of prepared buffered sample.

For example, measure 3 mL of sample and dilute it with 27 mL of sodium acetate solution. Pour 25 mL of this solution into a 50-mL beaker.



13. Dilute each unknown 10:1 with sodium acetate solution before measurement.

14. Place a stir bar in the beaker and set the beaker on a magnetic stirrer. Stir at a moderate speed.

15. Rinse the electrode with deionized water and blot dry.

16. Place the electrode into the sample. When the reading is stable, record the value.

Electrode assembly

1. Remove the cap from the electrolyte cartridge.
2. Visually inspect the Luer tip of the electrolyte cartridge. If air is present, rotate the feed-screw counter-clockwise until gel expels the air and fills the tip.
3. Fit the cartridge outlet tube firmly onto the inlet tube of the electrode body (Figure 24).

Figure 24 Attach the outlet tube

4. Place the dispenser unit over the electrolyte cartridge. Screw the dispenser unit onto the electrode body until reaching the stop. Do not over tighten.
5. Dispense the electrolyte gel by pressing the pump button. Repeat this procedure until gel is visible at the reference outlet (Figure 25).

Figure 25 Dispense the electrolyte gel

6. Rinse the electrode with deionized water. Do not scratch the crystal.
7. To remove an empty cartridge, unscrew the dispenser unit and rotate the cartridge counterclockwise while gently pulling it out of the electrode.

8. Connect the BNC connector of the electrode to the BNC connector on the meter (Figure 26).

Figure 26 BNC connector

Note: *One BNC and one 5-pin connector are on the back of the meter. Choose the BNC for the fluoride electrode. Disconnect the pH electrode from the 5-pin connector when using the BNC connector.*

Condition the electrode

Condition and store the electrode in 1 mg/L Fluoride standard storage with Ionic Strength Adjuster for 15 to 30 minutes.

For electrode storage procedures, refer to the Fluoride Electrode Instruction Manual.

Clean the Lanthanum Fluoride Crystal

It may be necessary to clean the LaF crystal on the sensing tip of the probe if it becomes covered with organic film or buildup.

1. Put a small amount of fluoride toothpaste on a soft toothbrush or cloth.
2. Gently rub the LaF crystal with the toothpaste using a circular motion. Rub until the film is removed.
3. Thoroughly rinse the probe with deionized water and blot dry. Verify the crystal is clean. If not, repeat cleaning and rinsing until it is clean.
4. If the crystal becomes contaminated by oil, grease, or fingerprints, soak for a few minutes in isopropyl alcohol then rinse with deionized water.

Interferences

Table 397 Interfering substances

Interfering substance	Interference level
Cations	Do not interfere
Cl^-, Br^-, SO_4^{2-}, HCO_3^-, PO_4^{3-}, acetate	Do not interfere
OH^- (Hydroxyl ions)	Interferes: refer to *pH Effects*. Some ions, such as CO_3^{2-} or PO_4^{3-}, make the sample more basic, which increases OH^- interference, but do not directly interfere with the electrode operation.
CO_3^{2-} or PO_4^{3-}	Can make the sample more basic and increase OH^-

pH Effects

In solutions with a pH below 5, hydrogen ion complexes some of the fluoride ions, forming the undissociated acid HF and the ion HF_2^-. Figure 27 shows the proportion of free fluoride ion in acid solutions.

If the background ionic strength is high and constant in comparison with the ion being measured, the activity coefficient is constant and activity is directly proportional to ion concentration. Total ionic strength adjustor is added to standards and samples to make the background ionic strength high, decomplex fluoride, and adjust the solution pH to 5.0–5.5.

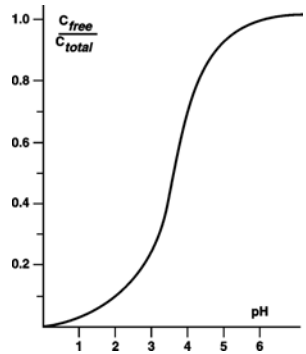


Figure 27 Ratio of free F^- in acid solutions

Sample collection, preservation and storage

- Collect samples in plastic bottles.
- Samples may be stored up to 28 days.

Accuracy check

Standard additions method (sample spike)
To verify measurement accuracy, perform a standard addition spike on the sample. The spike should roughly double the measured concentration without significantly diluting the sample.

To perform a standard addition sample:

1. Use the *Spike volumes* table to determine the concentration and volume of standard to spike the sample. The volume of sample transferred must be accurate.
2. Add the amount and concentration specified in the *Spike volumes* table to the sample.
3. After adding the standard, proceed with the calculations. Results from 90-110% recovery are considered acceptable. Calculate percent recovery as follows:

$$\% \text{ Recovery} = \frac{100(X_s - X_u)}{K}$$

Where:
X_s = measured value for spiked sample in mg/L

X_u = measured value for unspiked sample adjusted for dilution by the spike, in mg/L

K = known value of the spike in the sample in mg/L

Calculations

1. $X_u = \frac{X_i \times V_u}{V_u + V}$

Where:

X_i = measured value of unspiked sample in mg/L

V_u = volume of separate unspiked portion in mL

V = volume of spike in mL

2. $K = \frac{C \times V}{V_u + V}$

Where:

C = concentration of standard used in spike in mg/L

V = volume of spike in mL

V_u = volume of separate portion before spike in mL

3. Final calculation plugging in Xu and K: $\%\ \text{Recovery} = \frac{100(X_s - X_u)}{K}$

Example:

A sample was analyzed and read 5.0 mg/L F^-. As directed in the *Spike volumes* table, a 1.0-mL spike of 100-mg/L F^- standard was added to another 25-mL sample, giving a final standard addition result of 8.75 mg/L.

Calculate the percent recovery as follows:

1. $X_u = \frac{5.0\ \text{mg/L} \times 25\ \text{mL}}{25\ \text{mL} + 1\ \text{mL}} = 4.81\ \text{mg/L}$

2. $K = \frac{100\ \text{mg/L} \times 1\ \text{mL}}{25\ \text{mL} + 1\ \text{mL}} = 3.85\ \text{mg/L}$

3. $\%R = \frac{100 \times (X_s - X_u)}{K} = \frac{100 \times (8.75 - 4.81)}{3.85} = 102.3\ \%\ \text{Recovery}$

Table 398 Spike volumes

Measured Sample Concentration (mg/L)	Measured Sample Volume (mL)	Standard Concentration (mg/L)	Standard Volume (mL)
0.1–0.6	25	100	0.1
0.6–1.0	25	100	0.2
1.0–1.5	25	100	0.3
1.5–3.0	25	100	0.5
3–6	25	100	1.0
6–10	25	100	2.0
10–15	25	100	3.0
15–25	25	1000	0.5
25–35	25	1000	0.7
35–50	25	1000	1.0
50–100	25	1000	2.0

Summary of method

The fluoride electrode consists of a sensing Lanthanum Fluoride element bonded into an epoxy body. When the sensing element contacts fluoride ions in a solution, a potential develops across the sensing element. The potential is proportional to the level of fluoride ions present. The potential is measured against a constant reference potential with a pH/mV meter or ISE meter.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Fluoride Standard Solutions:			
1.00-mg/L F or	varies	500 mL	29149
10.00-mg/L F or	varies	500 mL	40520
100.0-mg/L F	varies	500 mL	35949
Potassium Chloride Reference Electrolyte			
Gel Cartridges	varies	2/pkg	2546902
Sodium Acetate, ACS	varies	454 g	17801H
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL, polypropylene	3	each	108041
Bottle, wash, 500-mL	1	each	62011
Cylinder, graduated, 25-mL, poly	3	each	108140
Fluoride Combination Electrode, BNC, w/ filling solution	1	each	5192800
*sens**ion*** 2 Portable pH/ISE Meter	1	each	5172500
OR			
*sens**ion*** 4 Laboratory pH/ISE Meter	1	each	5177500
Stir Bar, 7/8 x 3/16 in. (22.2 x 4.8 cm)	4	each	4531500
Select one based on available voltage:			
Stirrer, electromagnetic, 115 VAC, with stand and stir bar	1	each	4530001
Stirrer, electromagnetic, 230 VAC, with stand and stir bar	1	each	4530002
Tensette pipet 1.0–10.0 mL	1	each	1970010
Class A, 25 mL volumetric pipet	1	each	1451540
Safety bulb pipet filler	1	each	1418900
Class A, 1000 mL volumetric flask	1	each	1457453

Optional apparatus

Description	Unit	Catalog number
Electrode Washer	each	2704700
Pipet, TenSette, 0.1 to 1. 0 mL	each	1970001
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	2185696

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Fluoride in Drinking Water

DOC316.53.01237

USEPA[1] Direct Measurement ISE Method **Method 8323**

0.1 to 10.0 mg/L F^- **Powder Pillow or TISAB Solution**

Scope and Application: Drinking water

[1] USEPA equivalent method

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 399 Instrument-specific information

Meter	Electrode
*sens**ion***™ 4 meters	5192800
*sens**ion***™ 2 meters[1]	5192800

[1] The user must construct the calibration curve with the *sens**ion*** 2 meter.

Before starting the test:

Refer to the meter user manual for meter operation. Refer to electrode manual for electrode maintenance and care.
For USEPA reporting, replace the standards in step 1 with a 0.5-mg/L, 1.0-mg/L and 2.0-mg/L fluoride standard solution to calibrate the electrode.
Prepare the electrode. Refer to *Electrode assembly* and *Condition the electrode* in this procedure.

Collect the following items:

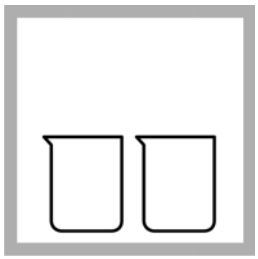
Description	Quantity
Fluoride ISA buffer pillows (TISAB)	1
OR	
Fluoride ISA solution, concentrated (TISAB)	5.0 mL
Sodium acetate, ACS	varies
Fluoride standard solutions:	
1.00-mg/L F	varies
2.00-mg/L F or	varies
0.5 mg/L F (USEPA)	varies
Potassium chloride reference electrolyte gel cartridges	varies
Water, deionized	varies

Collect the following items: (continued)

Description	Quantity
Beaker, 50-mL, polypropylene	3 or 4 (USEPA)
Bottle, wash, 500-mL	1
Cylinder, graduated, 25-mL, polypropylene	3
Platinum series fluoride combination electrode, BNC	1
*sens**ion***™2 Portable pH/ISE Meter. or *sens**ion***™4 Laboratory pH/ISE Meter	1
Stir Bar, 7/8 X 3/16 in. (22.2 x 4.8 cm)	2 or 3 (USEPA)
Stirrer, electromagnetic with stand and stir bar	1

See *Consumables and replacement items* for reorder information.

Fluoride in drinking water, powder pillows method

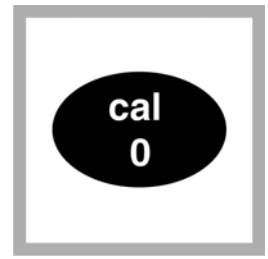


1. In 50-mL beakers, prepare two 25-mL standard solutions of 1-mg/L and 10-mg/L F^-.

2. Add the contents of one Fluoride Total Ionic Strength Adjustment Buffer (TISAB) Powder Pillow to each standard. Stir to dissolve.

3. Turn the meter on. Set the electrode type to BNC. Set the units to mg/L. Press **CAL**.

Refer to the meter user manual for details.



4. Place the beaker with the 1-mg/L standard on a magnetic stirrer. Stir at a moderate rate.

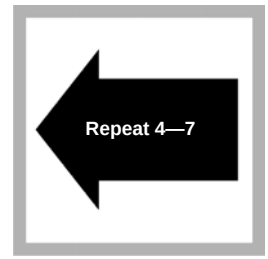
5. Place the electrode into the standard.

6. Edit the display to show the concentration of the standard.

Press **ENTER** to accept the concentration.

7. The display will show **Stabilizing** until the measurement is complete.

Remove the electrode from the standard solution Rinse with deionized water and blot dry.



8. Repeat steps 4–7 for the 10-mg/L and 0.5 mg/L (USEPA) standard.

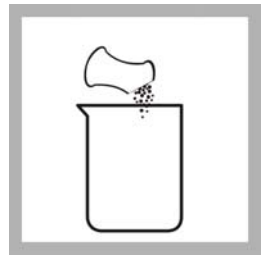
After the last standard is measured, store the calibration in the meter. Refer to the meter user manual for details.

Fluoride in drinking water, powder pillows method (continued)

9. Transfer 25 mL of the sample to a 50-mL beaker. Add a stir bar to the beaker. Place the beaker on a magnetic stirrer and stir at a moderate rate.

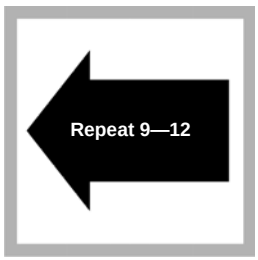
10. Remove the electrode from the standard solution. Rinse it with deionized water and blot dry. Place it into the sample.

The sample should be the same temperature as the standards, ±1 °C.



11. Add the contents of one TISAB Powder Pillow. Stir to dissolve.

12. After the measurement is stable, record or store the measurement value.

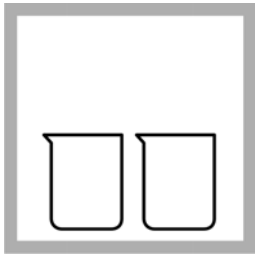


13. Repeat steps 9 through 12 for each sample.

14. Remove the electrode after reading the last sample. Rinse the electrode. Store in a fluoride standard of similar concentration to the sample that will be analyzed next.

Refer to the electrode manual for more information on electrode storage.

Fluoride in drinking water, liquid TISAB solution method

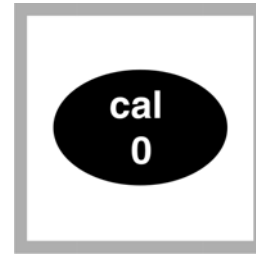


1. In 50-mL beakers, prepare two 25-mL standard solutions of 1-mg/L and 10-mg/L F^-.

2. Add the contents of 5 mL concentrated Fluoride Total Ionic Strength Adjustment Buffer (TISAB) per 25 mL of standard. Stir to mix.

3. Turn the meter on. Set the electrode type to BNC. Set the units to mg/L. Press **CAL**.

Refer to the meter user manual for details.



4. Place the beaker with the 1-mg/L standard on a magnetic stirrer. Stir at a moderate rate.

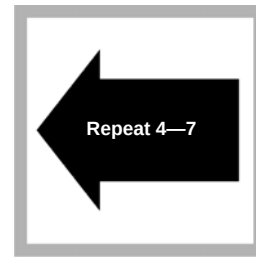
5. Place the electrode into the standard.

6. Edit the display to show the concentration of the standard.

Accept the concentration.

7. The display will show **Stabilizing** until the measurement is complete.

Remove the electrode from the standard solution Rinse with deionized water and blot dry.



8. Repeat steps 4–7 for the 10-mg/L standard.

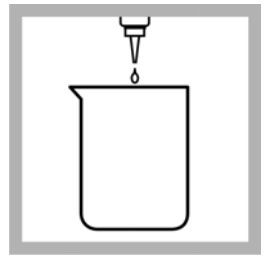
After the last standard is measured, store the calibration in the meter. Refer to the meter user manual for details.

Fluoride in drinking water, liquid TISAB solution method (continued)

9. Transfer 25 mL of the sample to a 50-mL beaker. Add a stir bar to the beaker. Place the beaker on a magnetic stirrer and stir at a moderate rate.

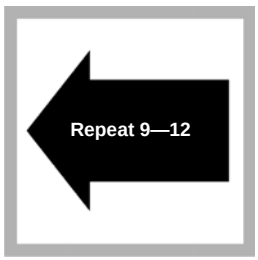
10. Remove the electrode from the standard solution. Rinse it with deionized water and blot dry. Place it into the sample.

The sample should be the same temperature as the standards, ±1 °C.



11. Add 5 mL concentrated liquid TISAB to the sample. Stir to mix.

12. After the measurement is stable, record or store the measurement value.



13. Repeat steps 9 through 12 for each sample.

14. Remove the electrode after reading the last sample. Rinse the electrode. Store in a fluoride standard of similar concentration to the sample that will be analyzed next.

Refer to the electrode manual for more information on electrode storage.

Electrode assembly

1. Remove the cap from the electrolyte cartridge.
2. Visually inspect the Luer tip of the electrolyte cartridge. If air is present, rotate the feed-screw counter-clockwise until gel expels the air and fills the tip.
3. Fit the cartridge outlet tube firmly onto the inlet tube of the electrode body (Figure 28).

Figure 28 Attach the outlet tube

4. Place the dispenser unit over the electrolyte cartridge. Screw the dispenser unit onto the electrode body until reaching the stop. Do not over tighten.
5. Dispense the electrolyte gel by pressing the pump button. Repeat this procedure until gel is visible at the reference outlet (Figure 29).

Figure 29 Dispense the electrolyte gel

6. Rinse the electrode with deionized water. Do not scratch the crystal.
7. To remove an empty cartridge, unscrew the dispenser unit and rotate the cartridge counterclockwise while gently pulling it out of the electrode.

8. Connect the BNC connector of the electrode to the BNC connector on the meter (Figure 30).

Figure 30 BNC connector

Note: *One BNC and one 5-pin connector are on the back of the meter. Choose the BNC for the fluoride electrode. Disconnect the pH electrode from the 5-pin connector when using the BNC connector.*

Condition the electrode

Condition and store the electrode in 1 mg/L Fluoride standard storage with Ionic Strength Adjuster for 15 to 30 minutes.

For electrode storage procedures, refer to the Fluoride Electrode Instruction Manual.

Clean the Lanthanum Fluoride Crystal

It may be necessary to clean the LaF crystal on the sensing tip of the probe if it becomes covered with organic film or buildup.

1. 1. Put a small amount of fluoride toothpaste on a soft toothbrush or cloth.
2. Gently rub the LaF crystal with the toothpaste using a circular motion. Rub until the film is removed.
3. Thoroughly rinse the probe with deionized water and blot dry. Verify the crystal is clean. If not, repeat cleaning and rinsing until it is clean.
4. If the crystal becomes contaminated by oil, grease, or fingerprints, soak for a few minutes in isopropyl alcohol then rinse with deionized water.

Interferences

Table 400 Interfering substances

Interfering substance	Interference level
Cations	Do not interfere
Cl^-, Br^-, SO_4^{2-}, HCO_3^-, PO_4^{3-}, acetate	Do not interfere
OH^- (Hydroxyl ions)	Interferes: refer to
CO_3^{2-} or PO_4^{3-}	Make the sample more basic and increase OH^-

pH Effects

In solutions with a pH below 5, hydrogen ion complexes some of the fluoride ions, forming the undissociated acid HF and the ion HF_2^-. Figure 31 shows the proportion of free fluoride ion in acid solutions.

If the background ionic strength is high and constant in comparison with the ion being measured, the activity coefficient is constant and activity is directly proportional to ion concentration. Total ionic strength adjustor is added to standards and samples to make the background ionic strength high, decomplex fluoride, and adjust the solution pH to 5.0–5.5.

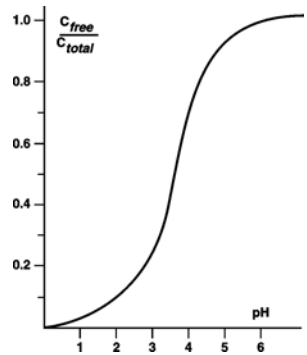


Figure 31 Ratio of free F^- in acid solutions

Sample collection, preservation and storage

- Collect samples in plastic bottles.
- Samples may be stored up to 28 days.

Accuracy check

Checking electrode response

To verify measurement accuracy, measure the electrode potential of two fluoride standard solutions that are one decade apart in concentration. For example, use 1-mg/L and 10-mg/L standards to bracket an expected sample concentration of 3 mg/L. The two standards should have mV potentials that are 58 ± 3 mV apart at 25 °C. Both solutions must be greater than 0.2 mg/L F^-.

Checking calibration accuracy

To verify calibration accuracy, measure the concentration of a known standard (e.g., 2.00 mg/L) within the calibration range.

Checking the accuracy of the sample reading

To verify sample measurement accuracy, add a spike of standard fluoride solution with a TenSette® or volumetric pipet. Use the *Spike Volumes* table and the formulas in.

Table 401 Spike Volumes

Measured sample Concentration	Volume & Concentration of F^- Standard to Add		C x V
	V	C	
0.6–1 mg/L	0.3 mL of...	...100-mg/L	30
1–2 mg/L	0.5 mL of...	...100-mg/L	50
3–6 mg/L	1.0 mL of...	...10-mg/L	100

Percent recovery

To calculate the percent recovery (only applicable if sample volume is 25 mL):

$$M = S \times 25 + C \times V$$

$$E = \frac{M}{25 + V}$$

$$R = \frac{A}{E} \times 100\%$$

Where:

M = calculated mass of fluoride present after the spike (micrograms)
S = mg/L of F^- in sample (before spike)
C = concentration of standard used for spiking (mg/L)
V = spike volume from the *Spike Volumes* table (mL)
E = expected concentration after spiking (mg/L)
R = percent recovery (should be 95–100%)
A = actual reading on meter after spike (mg/L F^-)

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
*sens**ion*** 4	1.6 mg/L	1.595–1.605 mg/L
*sens**ion*** 2		

Summary of method

The fluoride electrode consists of a sensing Lanthanum Fluoride element bonded into an epoxy body. When the sensing element contacts fluoride ions in a solution, a potential develops across the sensing element. The potential is proportional to the level of fluoride ions present. The potential is measured against a constant reference potential with a pH/mV meter or ISE meter.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Fluoride ISA buffer pillows	1	100/pkg	258999
OR			
Fluoride ISA solution	5 mL	3.78 L	2829017
Fluoride Standard Solutions:			
1.00-mg/L	varies	500 mL	29149
2.00-mg/L	varies	500 mL	40520
10.0-mg/L	varies	500 mL	35949
Potassium Chloride Reference Electrolyte Gel Cartridges	varies	2/pkg	2546902
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL, polypropylene	3	each	108041
Bottle, wash, 500-mL	1	each	62011
Cylinder, graduated, 25-mL, poly	3	each	108140
Fluoride Combination Electrode, BNC, w/ filling solution	1	each	5192800
*sens**ion*** 2 Portable pH/ISE Meter	1	each	5172500
OR			
*sens**ion*** 4 Laboratory pH/ISE Meter	1	each	5177500
Stir Bar, $^{7}/_{8}$ x $^{3}/_{16}$ in. (22.2 x 4.8 cm)	1	each	4531500
Select one based on available voltage:			
Stirrer, electromagnetic, 115 VAC, with stand and stir bar	1	each	4530001
Stirrer, electromagnetic, 230 VAC, with stand and stir bar	1	each	4530002

Optional apparatus

Description	Unit	Catalog number
Electrode Washer	each	2704700
Pipet, TenSette, 0.1 to 1. 0 mL	each	1970001
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	2185696

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Edition 7

Nitrate

DOC316.53.01238

Direct Measurement ISE Method **Method 8358**

0.1 to 100.0 mg/L NO_3–N **Powder Pillow or TISAB Solution**

Scope and Application: Water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 402 Instrument-specific information

Meter	Electrode
*sens**ion***™ 4 meters	5192000
*sens**ion***™ 2 meters	5192000

Before starting the test:

Refer to the meter user manual for meter operation. Refer to electrode manual for electrode maintenance and care.
Prepare the electrode. Refer to *Electrode assembly* and *Nitrate half-cell preparation* in this procedure.
When using the electrode for the first time, condition the reference electrode for eight hours in a 100 mg/L nitrate-nitrogen standard
Temperature variation causes inaccurate measurements. Calibration and sample measurements should be made at the same temperature ± 1 °C.
The stable sample reading is the nitrate-nitrogen (NO_3^-–N) concentration expressed as elemental nitrogen (N). The results can be expressed as mg/L nitrate (NO_3^-) by multiplying the results by 4.4.

Collect the following items:

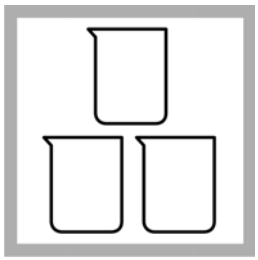
Description	Quantity
Ammonium sulfate reference electrolyte gel cartridge	1
Nitrate half-cell internal filling solution	0.5 mL
Nitrate ISA powder pillows	1 per sample or standard
OR	
Nitrate ISA solution	25 mL per sample or standard
Nitrate-Nitrogen standard solutions:	
1-mg/L as NO_3^-–N	25 mL
10-mg/L as NO_3^-–N	25 mL

Collect the following items: (continued)

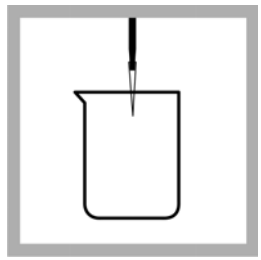
Description	Quantity
100-mg/L as NO_3^-–N	25 mL
Water, deionized	varies
Beaker, 100-mL, polypropylene	4
Bottle, wash, 500-mL	1
Cylinder, graduated, 25-mL, polypropylene	4
Combination Nitrate Electrode, BNC	1
pH/ISE meter, *sens**ion***™2 (portable) or *sens**ion***™4 (laboratory)	1
Stir Bar, 7/8 X 3/16 in. (22.2 x 4.8 cm)	4
Stirrer, electromagnetic with stand and stir bar	1
TenSette® pipet, 1.0–10.0 mL	1
OR	
25 mL Class A volumetric pipet	1
Safety bulb pipet filler	1
Membrane Tip, for nitrate electrode	1

See *Consumables and replacement items* for reorder information.

Nitrate-Nitrogen, powder pillow ISA method

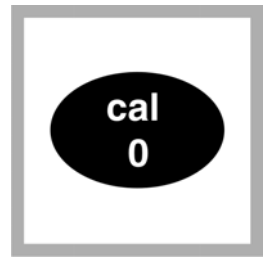


1. In 100-mL beakers, prepare three 50-mL standard solutions of 1, 10 and 100 mg/L NO_3^-–N.



2. Use a TenSette® Pipet to pipet 25 mL of standard into each beaker. Add the contents of one Ionic Strength Adjustor (ISA) powder pillow into each beaker to make the buffered standard.

Stir to dissolve.



3. Turn the meter on. Set the electrode type to BNC. Set the units to mg/L. Press **CAL**.

Refer to the meter user manual for details.

4. Add a stir bar to each beaker. Put the beaker with the 1-mg/L buffered standard on a magnetic stirrer. Stir at a moderate rate.

Starting with the lowest concentration standard reduces carry-over contamination and gives optimal electrode response.

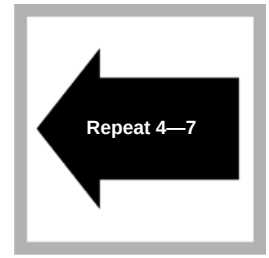
Nitrate-Nitrogen, powder pillow ISA method (continued)

5. Put the electrode into the 1.0 mg/L buffered standard.

6. Edit the display to show the concentration of the standard.

Press **ENTER** to accept the concentration.

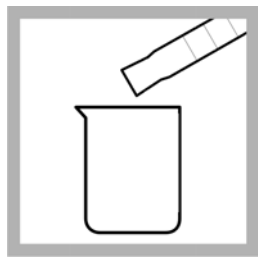
7. When the measurement stabilizes, remove the electrode from the standard solution Rinse with deionized water and blot dry.



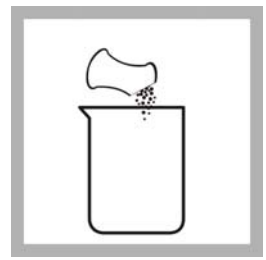
8. Repeat steps 4–7 for the 10-mg/L and 100 mg/L buffered standards.

After the last standard is measured, store the calibration in the meter. Refer to the meter user manual for details.

9. Remove the electrode from the standard solution. Rinse it with deionized water and blot dry.



10. Accurately measure 25.0 mL of sample into a 100-mL beaker.



11. Add the contents of one ISA powder pillow into the beaker with the sample to make the buffered sample. Stir to dissolve.

A white precipitate will form if chloride or other ions are present. This will not harm the electrode or interfere with the analysis.

12. Add a stir bar to the buffered sample. Put the buffered sample on a stirrer and stir at a moderate rate. Put the electrode into the buffered sample.

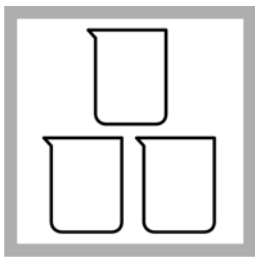
Nitrate-Nitrogen, powder pillow ISA method (continued)

13. Press **READ**. When the measurement stabilizes, record or store the value.

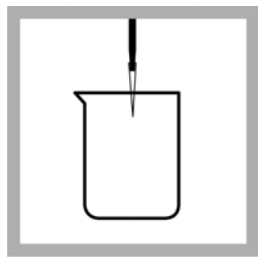
14. Remove the electrode after reading the sample. Rinse the electrode. Store in a nitrate standard (without ISA) of similar concentration to the sample that will be analyzed next.

Refer to the electrode manual for more information on electrode storage.

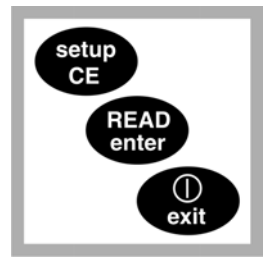
Nitrate-Nitrogen, liquid ISA method



1. In 100-mL beakers, prepare three 50-mL standard solutions of 1, 10 and 100 mg/L NO_3^-–N.

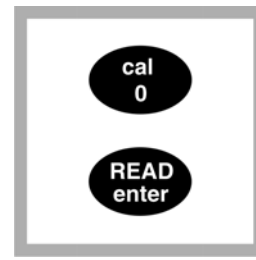


2. Use a TenSette® Pipet to pipet 25 mL of standard into each beaker. Pipet 25 mL of liquid Ionic Strength Adjustor (ISA) into each beaker to make the buffered solution.



3. Turn the meter on. Set the electrode type to BNC. Set the units to mg/L. Press **CAL**.

Refer to the meter user manual for details.



4. Add a stir bar to each beaker. Put the beaker with the 1-mg/L standard on a magnetic stirrer. Stir at a moderate rate.

Starting with the lowest concentration standard reduces carry-over contamination and gives optimal electrode response.

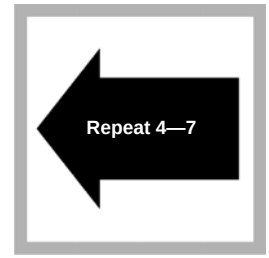
Nitrate-Nitrogen, liquid ISA method (continued)

5. Put the electrode into the 1.0 mg/L buffered standard.

6. Edit the display to show the concentration of the standard.

Press **ENTER** and accept the concentration.

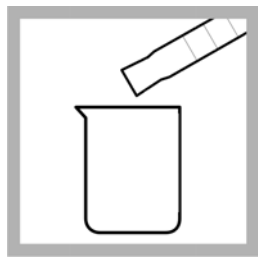
7. When the measurement stabilizes, remove the electrode from the standard solution Rinse with deionized water and blot dry.



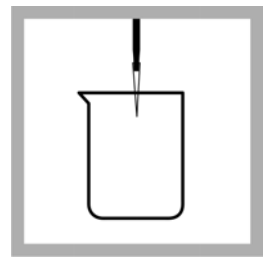
8. Repeat steps 4–7 for the 10-mg/L and 100 mg/L buffered standard.

After the last standard is measured, store the calibration in the meter. Refer to the meter user manual for details.

9. Remove the electrode from the buffered standard. Rinse it with deionized water and blot dry.



10. Accurately measure 25.0 mL of sample into a 100-mL beaker.



11. Pipet 25.0 mL of liquid ISA into the beaker with the sample.

A white precipitate will form if chloride or other ions are present. This will not harm the electrode or interfere with the analysis.

12. Add a stir bar to the buffered sample. Put the buffered sample on a stirrer and stir at a moderate rate. Put the electrode into the buffered sample.

Nitrate-Nitrogen, liquid ISA method (continued)

13. Press **READ** when the measurement stabilizes to record or store the value.

14. Remove the electrode after reading the sample. Rinse the electrode. Store in a nitrate standard of similar concentration to the sample that will be analyzed next.

Refer to the electrode manual for more information on electrode storage.

Nitrate half-cell preparation

1. Condition the nitrate membrane tip by immersing the membrane tip in beaker with 100 mg/L NO_3–N solution for approximately one hour prior to use. When using the electrode for the first time, condition the reference electrode for eight hours in a 100 mg/L nitrate–nitrogen standard.
2. Clean all gel from the electrode tip and glass stem. Rinse with Deionized water and dry completely with a soft paper towel.
3. Use a soft paper towel soaked with isopropyl or rubbing alcohol to wipe the glass stem. Dry the stem with a dry, soft paper towel. Make sure that alcohol does not get into the glass stem.
4. Use the provided syringe to fill the membrane to with the electrode filling solution. Fill with solution to the top of the tip. Alternatively, fill the glass stem as shown in the electrode manual. **Do not fill the stem completely or the pressure will cause the membrane tip to fall off.**
5. Use a gentle, twisting action to carefully slide a clean, dry nitrate membrane tip over the glass stem until the end of the stem rests midway through the Nitrate Membrane Tip and some resistance is met. Leave a $^1/_8$-inch gap between the tip and the electrode body.
6. Shake the electrode as if shaking down mercury in a thermometer to make sure the Nitrate Electrode Filling Solution contacts the end of the nitrate membrane tip. Verify that air bubbles are not present in the tip.

Electrode assembly

1. Remove the cap from the electrolyte cartridge.
2. Visually inspect the Luer tip of the electrolyte cartridge. If air is present, rotate the feed-screw counter-clockwise until gel expels the air and fills the tip.

3. Fit the cartridge outlet tube firmly onto the inlet tube of the electrode body (*Attach the outlet tube*).

Figure 32 Attach the outlet tube

4. Place the dispenser unit over the electrolyte cartridge. Screw the dispenser unit onto the electrode body until reaching the stop. Do not over tighten.
5. Dispense the electrolyte gel by pressing the pump button. Repeat this procedure until gel is visible at the reference outlet (*Dispense the electrolyte gel*). If readings become erratic make sure that the electrolyte gel is completely purged through the reference line.

Figure 33 Dispense the electrolyte gel

6. Rinse the electrode with deionized water. Do not scrub the electrode tip.
7. Connect the BNC connector of the electrode to the BNC connector on the meter (*BNC connector*).

Figure 34 BNC connector

Interferences

For the nitrate electrode, the major interferences include perchlorate, iodide, nitrite, bromide and chloride. The addition of Nitrate ISA will eliminate most of these interferences. The highest level of chloride the ISA can accommodate is 40 mg/L Cl^-. Concentrations greater than 40 mg/L Cl^- in the sample will cause false high nitrate values. For more information on selectivity coefficients without ISA, refer to the Electrode Characteristics section of the electrode manual.

Sample collection, preservation and storage

- Collect samples in clean glass or plastic bottles. Start nitrate analysis promptly after sampling.
- If storage is necessary, store for up to 24 hours at 4 °C or lower (0–6 °C as per USEPA MUR March 2007).
- For longer storage periods, adjust sample pH to 2 or less with 2 mL sulfuric acid per liter of sample. Sample refrigeration is still necessary. When sample is preserved with acid, NO_3^- and NO_2^- cannot be determined.
- Before testing a preserved sample, warm to room temperature, then neutralize to approximately pH 7 with 5.0 N Sodium Hydroxide Standard Solution.
- Do not use mercury compounds as preservatives.
- Correct the test result for volume additions.

Accuracy check

Checking electrode response

To verify electrode response, measure the electrode potential (in mV) of two Nitrate–Nitrogen standard solutions one decade apart in concentration, bracketing the expected sample concentration. For example, use 10 and 100 mg/L Nitrate–Nitrogen Standard Solutions to bracket an expected sample concentration of 30 mg/L. The two solutions should have potentials (in mV) that are 58 ± 3 mV apart at 25 °C. Both solutions must be above 5 mg/L NO_3^-–N.

Checking calibration accuracy

To verify calibration accuracy, measure the concentration of a known standard within the calibration range.

Checking the accuracy of the sample reading

To verify sample measurement accuracy, add a spike of standard NO_3^-–N solution with a TenSette® or volumetric pipet. Use the *Spike volumes* table and the formulas in *Percent recovery*.

Table 403 Spike volumes

Measured sample Concentration	Volume & Concentration of F^- Standard to Add		C x V
	V	C	
1–2 mg/L	0.5 mL of	100 mg/L	50
3–6 mg/L	1.0 mL of	100 mg/L	100
7–15 mg/L	0.3 mL of	1000 mg/L	300
15–30 mg/L	0.5 mL of	1000 mg/L	500

Percent recovery

To calculate the percent recovery (only applicable if sample volume is 25 mL):

$$M = S \times 25 + C \times V$$

$$E = \frac{M}{25 + V}$$

$$R = \frac{A}{E} \times 100\%$$

Where:

M = calculated mass of nitrate as nitrogen present after the spike (micrograms)
S = mg/L of NO_3^-–N in sample (before spike)
C = concentration of standard used for spiking (mg/L)
V = spike volume from the *Spike volumes* table (mL)
E = expected concentration after spiking (mg/L)
R = percent recovery (should be 95–100%)
A = actual reading on meter after spike (mg/L NO_3^-–N)

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
*sens**ion*** 4[1]	5.0 mg/L	4.56–5.44 mg/L
*sens**ion*** 2[1]		

[1] With a default stabilization criteria of 0.5 mV/min.

Summary of method

Nitrate ions are selectively absorbed by the ISE membrane, establishing a potential (voltage) that is proportional to the concentration of nitrate in the sample. This potential is compared to the constant potential of a reference electrode by measuring the potential of known standard. A calibration curve can be constructed to determine the concentration of nitrate in unknown samples.

The solvent-polymer membrane is a nitrate ion-exchanger in an inert polyvinyl chloride (PVC) plastic matrix. The nitrate electrode has an internal silver/silver chloride element, which establishes a fixed potential when in contact with the internal filling solution. The ion selective membrane undergoes ion exchange with nitrate in the sample, creating a potential across the membrane which varies with the amount of nitrate ion in the sample. This potential will decrease by about 58 mV for every tenfold increase in nitrate concentration in the linear operating range at 25 °C.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Nitrate ISA powder pillows	1	100/pkg	258999
Nitrate half-cell filling solution	varies	50 mL	4456369
Nitrate-Nitrogen Standard Solutions:			
1-mg/L	varies	500 mL	29149
10-mg/L	varies	500 mL	35949
100-mg/L	varies	500 mL	35949
1000-mg/L	varies	500 mL	35949
Ammonium Sulfate Reference Electrolyte Gel Cartridge	varies	2/pkg	2597102
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL, polypropylene	3	each	108041
Bottle, wash, 500-mL	1	each	62011
Cylinder, graduated, 25-mL, poly	3	each	108140
Nitrate Combination Electrode, Platinum series, BNC	1	each	5192000
*sens**ion*** 2 Portable pH/ISE Meter	1	each	5172500
OR			
*sens**ion*** 4 Laboratory pH/ISE Meter	1	each	5177500
Stir Bar, $^{7}/_{8}$ x $^{3}/_{16}$ in. (22.2 x 4.8 cm)	1	each	4531500
Select one based on available voltage:			
Stirrer, electromagnetic, 115 VAC, with stand and stir bar	1	each	4530001
Stirrer, electromagnetic, 230 VAC, with stand and stir bar	1	each	4530002
Pipet, TenSette, 0.1 to 1.0 mL	1	each	1970001
OR			
Class A 25 mL volumetric pipet	1	each	1451540
Safety bulb pipet filler	1	each	1465100
Membrane Tips, for nitrate electrode (replacement)	1	each	4613300

Optional reagents

Description	Unit	Catalog number
Nitrate Ionic Strength Adjustor, powder	454 g	4456301
Sodium Hydroxide Standard Solution, 5 N	100 mL	245032
Sulfuric Acid, ACS	500 mL	97949

Optional apparatus

Description	Unit	Catalog number
Pipet Tips, for 197001 TenSette Pipet	50/pkg	2185696
Scoop, measuring, 0.5 gram	each	90700
Scoop, measuring, 0.2 gram	each	63800

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Edition 7

Nitrate

DOC316.53.01239

Direct Measurement ISE Method **Method 8359**

0.04 to 4.00 mg/L NO_3–N **TISAB Solution**

Scope and Application: Drinking water

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 404 Instrument-specific information

Meter	Electrode
*sens**ion***™ 4 meters[1]	5192000

[1] Only *sension* 4 meters can be used for this analysis.

Before starting the test:

Refer to the meter user manual for meter operation. Refer to electrode manual for electrode maintenance and care.
When using the electrode for the first time, condition the reference electrode for eight hours in a 100 mg/L nitrate–nitrogen standard. After initial conditioning (before the first use or after long-term storage), condition the electrode for 60 minutes only, unless the electrode is stored dry for longer than four weeks. If the electrode is used daily, store the electrode in a nitrate standard having a concentration similar to the measured samples.
The low range nitrate test requires several conditioning steps and careful attention to temperature. Read through the procedure steps and the *Sample collection, preservation and storage* section before proceeding. The 2-hour conditioning step must be completed before the calibration step.
Sample concentration for this test must be 3.0 mg/L NO_3^-–N or less. Use a test strip to determine nitrate concentration . If the electrode is conditioned to a low nitrate-nitrogen concentration (as in this procedure), putting it in a concentrated NO_3^-–N sample (10 mg/L or higher) will swamp the electrode with nitrate ion and the 2-hour conditioning step must be repeated.
Note A: If the electrode is conditioned properly, the mV potential should not change more than 0.1 mV every five minutes in step 7. It may be necessary to press the electrolyte dispenser button again to stabilize the potential reading. If the potential drifts one direction more than 0.2 mV every 5 minutes, leave the electrodes in the 150-mL beaker until the drift has slowed. If the drift is higher than 0.1 mV per minute, the electrode should be conditioned longer in 100 mL of 0.040 mg/L standard. **Do not** put ISA into the standard.
Prepare the electrode. Refer to *Electrode assembly* and *Nitrate half-cell preparation* in this procedure.
Use identical amounts of ionic strength adjustor (ISA) in the standard beaker and in sample measurements. Use liquid ISA.
Temperature variation causes inaccurate measurements. Calibration and sample measurements should be made at the same temperature ± 1 °C.
The stable sample reading is the nitrate-nitrogen (NO_3^-–N) concentration expressed as elemental nitrogen (N). The results can be expressed as mg/L nitrate (NO_3^-) by multiplying the results by 4.4.

Collect the following items:

Description	Quantity
Ammonium sulfate reference electrolyte gel cartridge	1
Nitrate half-cell internal filling solution	0.5 mL
Nitrate ISA solution	25 mL per sample or standard
Nitrate Electrode Membrane Tip (replacement)	1
Pipet, TenSette, 0.1 to 1.0 mL	1
Pipet, TenSette, 1.0 to 10.0 mL	1
Nitrate-Nitrogen standard solutions:	
10-mg/L as NO_3^-–N	25 mL
100-mg/L as NO_3^-–N	25 mL
Water, deionized	varies
Beaker, 150-mL, polypropylene	3
Bottle, wash, 500-mL	1
Cylinder, graduated, 25-mL, polypropylene	3
Combination Nitrate Electrode, BNC	1
*sens**ion***™4 (laboratory)	1
Stir Bar, 7/8 X 3/16 in. (22.2 x 4.8 cm)	1
Stirrer, electromagnetic with stand and stir bar	1
Thermometer, Digital	1
100 mL plastic volumetric flask	2

See *Consumables and replacement items* for reorder information.

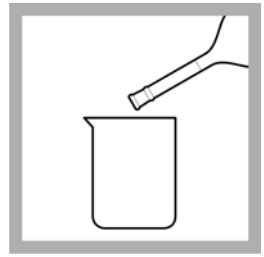
Nitrate-Nitrogen, liquid ISA method

1. Condition the electrode in 100 mg/L NO_3–N standard for one hour (refer to *Nitrate half-cell preparation* and Electrode Assembly in this procedure). Then condition the electrode in 0.010 mg/L NO_3–N for at least 2 hours.

To make 100 mL of 0.010 mg/L NO_3–N, use a TenSette Pipet to deliver 0.10 mL of 10 mg/L NO_3–N into a 100 mL volumetric flask and dilute to the mark. Mix well.

2. Use a TenSette® Pipet to pipet 5.0 mL of of nitrate liquid ISA into a plastic 100-mL volumetric flask. Bring to the 100-mL mark with deionized water.

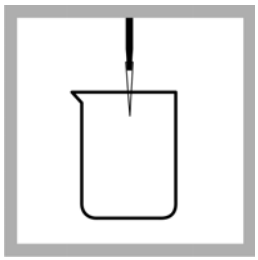
The deionized water must be at room temperature.



3. Pour the solution from the flask into a 150-mL beaker.

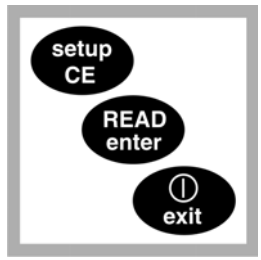
Add a large stir bar (50.8 x 7.9 mm) to the beaker, place the beaker on an electromagnetic stirrer and begin stirring at a moderate rate.

4. Remove the electrode from the 0.010 mg/L nitrate–nitrogen standard and rinse it well with deionized water. Place the electrode in the 150-mL beaker, submerging the tip below the solution surface.



5. Use a TenSette Pipet to add 0.4 mL of 10 mg/L nitrate–nitrogen standard solution to the solution in the beaker (this makes 100 mL of 0.04 mg/L nitrate–nitrogen).

Allow the electrode to condition for 30 minutes in this solution before proceeding.



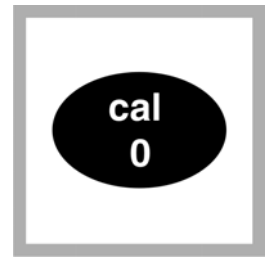
6. Turn the meter on. Press **SETUP**, set the electrode type to BNC and scroll to Stabilizing.

Press **ENTER** and edit the display to show 0.1 mg/min. Accept the value and **EXIT** the setup menu.

7. Press **ISE MV** until the mV potential shows on the display. Refer to *Before starting the test: Note A*.

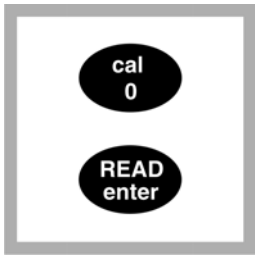
After the measurement is stable, record the potential value.

Measure the temperature of the standard (°C) with a lab grade thermometer. Record the temperature.



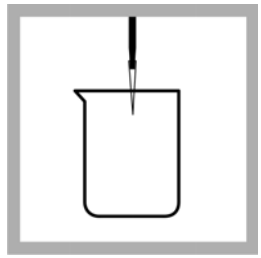
8. Press **ISE MV** to toggle to concentration units. Press **CAL** and scroll to mg/L. Press **ENTER** and accept the concentration units.

Nitrate-Nitrogen, liquid ISA method (continued)



9. Edit the display to show the concentration of the solution in the 150 mL beaker (0.040 mg/L). Refer to the *Low level nitrate calibration table*.

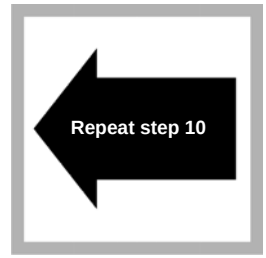
Press **ENTER** to accept the concentration.



10. When the measurement stabilizes, pipet the corresponding additional volume from the *Low level nitrate calibration table*. Wait the amount of time specified for step 2 in the table to allow the membrane to respond.

Enter the concentration in mg/L (0.080 mg/L). Accept the concentration.

If Error 2 occurs, use 0.08 mg/L as the first standard for this calibration. Then try conditioning in 0.04 mg/L NO_3^-–N solution for subsequent attempts.



11. Repeat step 10, adding the additional volumes of 10 mg/L and 100 mg/L NO_3^-–N standard from the *Low level nitrate calibration table* until all seven standards have been measured. Store the calibration and return to measurement mode.

12. Remove the electrode from the last standard, rinse well with deionized water, and blot dry.

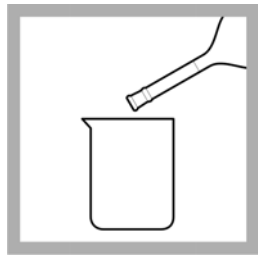
Save the solution in the 150-mL beaker with the 3.84 mg/L NO_3^-–N for later calibration checks.

Nitrate-Nitrogen, liquid ISA method (continued)

13. Accurately pipet 5 mL of nitrate liquid ISA into a Class A 100-mL volumetric flask. Bring to the mark with the sample being measured.

The sample must be at the same temperature as the standard solution in the 150-mL beaker used to perform the calibration.

A white precipitate will form if chloride or other ions that react with silver are present in solution. This will not hurt the electrode or interfere with the analysis.



14. Pour this 100 mL of sample into a 150-mL beaker. Add a magnetic stir bar, place the beaker on an electromagnetic stirrer. Stir at a moderate rate.

15. Put the electrode into the sample and press the electrolyte dispenser button once.

16. Wait 5–10 minutes to allow electrode to condition to the low level nitrate in solution.

Read the nitrate concentration from the display after it stabilizes.

This is the sample nitrate concentration expressed as elemental nitrogen (NO_3^-–N).

Note: *Record this value. Between uses, the electrode can be stored in the sample (if not an extreme pH). See electrode manual for details.*

Calibration

Make sure to measure millivolt potentials of all nitrate standards at the same temperature ± 0.5 °C. The sample and standards must be measured at the same temperature, ± 1 °C. This procedure keeps temperature error to a minimum by using a spiked additions method of calibration. Even so, care should be taken to use the 100-mg/L NO_3–N standard at room temperature.

Use a water bath slightly above room temperature (25 °C) to equilibrate the standard temperature and sample temperature before measuring mV potentials. Use a laboratory-grade thermometer to monitor the temperature. A one degree centigrade difference may result in as much as a 0.4 mV inaccuracy. This temperature variation will, in turn, decrease accuracy of concentration measurements.

Table 405 Low level nitrate calibration table

Step	Volume 10 mg/L NO_3^-–N standard added	Volume 100 mg/L NO_3^-–N standard added	Concentration	
			mg/L	Time
1	0.4 mL		0.040	30 min.
2	0.4 mL		0.080	10 min.
3	1.2 mL		0.20	5 min.
4		0.2 mL	0.40	5 min.
5		0.4 mL	0.80	5 min.
6		1.2 mL	1.96	5 min.
7		2.0 mL	3.84	5 min.

Nitrate half-cell preparation

1. Condition the nitrate membrane tip by immersing the membrane tip in beaker with 100 mg/L NO_3–N solution for approximately one hour prior to use.When using th electrode for the first time, condition the reference electrode for eight hours in a 100 mg/L nitrate–nitrogen standard.
2. Clean all gel from the electrode tip and glass stem. Rinse with Deionized water and dry completely with a soft paper towel.
3. Use a soft paper towel soaked in isopropyl or rubbing alcohol to wipe the glass stem. Dry the stem with a dry, soft paper towel. Make sure that alcohol does not get in the glass stem.
4. Use the provided syringe to fill the membrane to with the electrode filling solution. Fill with solution to the top of the tip. Alternatively, fill the glass stem as shown in the electrode manual. **Do not fill the stem completely or the pressure will cause the membrane tip to fall off.**
5. Use a gentle, twisting action to carefully slide a clean, dry nitrate membrane tip over the glass stem until the end of the stem rests midway through the Nitrate Membrane Tip and some resistance is met. Leave a $^1/_8$-inch gap between the tip and the electrode body.
6. Shake the electrode as if shaking down mercury in a thermometer to make sure the Nitrate Electrode Filling Solution contacts the end of the nitrate membrane tip. Verify that air bubbles are not present in the tip.

Electrode assembly

1. Remove the cap from the electrolyte cartridge.
2. Visually inspect the Luer tip of the electrolyte cartridge. If air is present, rotate the feed-screw counter-clockwise until gel expels the air and fills the tip.
3. Fit the cartridge outlet tube firmly onto the inlet tube of the electrode body.
4. Place the dispenser unit over the electrolyte cartridge. Screw the dispenser unit onto the electrode body until reaching the stop. Do not over tighten.
5. Dispense the electrolyte gel by pressing the pump button. Repeat this procedure until gel is visible at the reference outlet. If readings become erratic make sure that the electrolyte gel is completely purged through the reference line.
6. Rinse the electrode with deionized water. Do not scrub the electrode tip.
7. Connect the BNC connector of the electrode to the BNC connector on the meter.

Interferences

For the nitrate electrode, the major interferences include perchlorate, iodide, nitrite, bromide and chloride. The addition of Nitrate ISA will eliminate most of these interferences. The highest level of chloride the ISA can accommodate is 40 mg/L Cl^-. Concentrations greater than 40 mg/L Cl^- in the sample will cause false high nitrate values. For more information on selectivity coefficients without ISA, refer to the Electrode Characteristics section of the electrode manual.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Start nitrate measurements promptly after sampling. If storage is necessary, store for up to 24 hours at 4 °C or lower (as per the USEPA MUR in March 2007, the storage criteria changed from 4 °C to 0–6 °C).
- **Do not adjust sample pH**. This method is for low ionic strength use only. Adjusting the pH with acid will change the ionic strength and make this method unusable.

Accuracy check

Checking electrode response

To verify electrode response at these low levels of nitrate, the millivolt potential should decrease upon each addition of 100 mg/L NO3-N. Using the Low Level Nitrate-Nitrogen procedure, at least a 5.0 mV drop should be observed from step 1 to step 2 (0.040 mg/L to 0.080 mg/L NO3--N). Each additional spike should decrease the mV reading substantially from the previous change. If this is not the case, check the purity of the standard. If this is not the problem, use a new membrane.

Checking calibration accuracy

1. Pipet 1 mL liquid Nitrate ISA into a 100-mL volumetric flask.
2. Fill the 100-mL volumetric flask to the mark with 1.0 mg/L NO_3^-–N. Pour this solution in a 100-mL beaker and add a stir bar.
3. Put the beaker on an electromagnetic stirrer and measure the concentration of the solution with the calibrated electrode. The measurement should be 1.0 mg/L ± 0.1 mg/L.

Note: *The beaker containing the 3.0 mg/L NO_3^-–N standard used in the calibration may also be used as a check on the calibration. It should read close to 3.0 mg/L NO_3^-–N ± 0.1 mg/L.*

Checking the accuracy of the sample reading

To verify sample measurement accuracy, add a spike of standard NO_3^-–N solution with a TenSette® or volumetric pipet. Use the *Spike volumes of 100-mg/L standard* table and the formulas in *Percent recovery*.

Table 406 Spike volumes of 100-mg/L standard

Measured sample Concentration NO_3^-–N	Volume of 100 ppm Standard to Add	C x V
0.04 to 0.3	0.1 mL	10
0.30 to 0.60	0.3 mL	30
0.6 to 0.9	0.5 mL	50
0.9 to 3.0	1.0 mL	100

Percent recovery

To calculate the percent recovery:

$$M = S \times 100 + (C \times V)$$

$$E = \left[\frac{M}{100 + V}\right]$$

$$R = \frac{A}{E} \times 100\%$$

Where:

M = calculated mass of nitrate as nitrogen present after the spike (micrograms)
S = mg/L of NO_3^-–N in sample (before spike)
C = concentration of standard used for spiking (mg/L)
V = spike volume from the *Spike volumes of 100-mg/L standard* table (mL)
E = expected concentration after spiking (mg/L)
R = percent recovery (should be 95–100%)
A = actual reading on meter after spike (mg/L NO_3^-–N)

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
*sens**ion*** 4[1]	0.1 mg/L	0.095–0.1005 mg/L

[1] With a stabilization criteria of 0.1 mV/min

Summary of method

Nitrate ions are selectively absorbed by the ISE membrane, establishing a potential (voltage) that is proportional to the concentration of nitrate in the sample. This potential is compared to the constant potential of a reference electrode by measuring the potential of known standard. A calibration curve can be constructed to determine the concentration of nitrate in unknown samples.

The solvent-polymer membrane is a nitrate ion-exchanger in an inert polyvinyl chloride (PVC) plastic matrix. The nitrate electrode has an internal silver/silver chloride element, which establishes a fixed potential when in contact with the internal filling solution. The ion selective membrane undergoes ion exchange with nitrate in the sample, creating a potential across the membrane which varies with the amount of nitrate ion in the sample. This potential will decrease by about 58 mV for every tenfold increase in nitrate concentration in the linear operating range at 25 °C.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ammonium Sulfate Reference Electrolyte Gel Cartridge	varies	2/pkg	2597102
Nitrate Electrode Membrane Tips	varies	16/pkg	4613300
Nitrate-Nitrogen Standard Solutions 10 mg/L as NO_3^-–N	varies	500 mL	30749
Nitrate ISA liquid	varies	500 mL	2488349
Nitrate half-cell filling solution	varies	50 mL	4456369
Nitrate-Nitrogen Standard Solution 100 mg/L NO_3^-–N	varies	500 mL	194749
Nitrate test strips	varies	25/pkg	2745425
Water, deionized	varies	4 L	27256

Required apparatus

Description	Unit	Catalog number
Beaker, 150-mL, polypropylene	each	108044
Bottle, wash, 500-mL	each	62011
Pipet, TenSette, 0.1–1.0 m	each	1970001
Flask, volumetric, poly, 100 mL	each	1406042
Thermometer, Digital	each	2630600
Flask, volumetric, 100 mL, polypropylene	each	1406041
Nitrate Combination Electrode, Platinum series, BNC	each	5192000
*sens**ion*** 4 Laboratory pH/ISE Meter	each	5177500
Stir Bar, $^7/_8$ x $^3/_{16}$ in. (22.2 x 4.8 cm)	each	4531500
Stir Bar, $1^1/_8$ x $^3/_{10}$ in. (28.6 x 7.9 cm)	each	2095355
Select one based on available voltage:		
Stirrer, electromagnetic, 115 VAC, with stand and stir bar	each	4530001
Stirrer, electromagnetic, 230 VAC, with stand and stir bar	each	4530002
Nitrate Electrode Membrane Tips (replacements)	6/pkg	4613300

Optional reagents

Description	Unit	Catalog number
Nitrate Ionic Strength Adjustor, powder	454 g	4456301
Nitrate Nitrogen Standard Solutions 1 mg/L as NO_3^-–N	500 mL	204649

Optional apparatus

Description	Unit	Catalog number
Beaker, 100 mL, polypropylene	each	108042
Pipet Tips, for TenSette Pipet	50/pkg	2185696
Water Bath, circulating	each	2616300
Pipet, Mohr, 2.00 mL, glass	each	2093636

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Edition 7

Nitrogen, Ammonia

DOC316.53.01235

USEPA[1] Direct Measurement ISE Method[2] **Method 10001**

0.1 to 10.0 mg/L NH_3-N **ISE Electrode**

Scope and Application: For wastewater

[1] USEPA Accepted for reporting wastewater analyses

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, Method 4500NH3E (with distillation). Manual distillation may not be required if comparability data on representative samples in company files show the distillation is not necessary. Manual distillation will be required to resolve any controversies.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 407 Instrument-specific information

Meter	Electrode
*sens**ion*™ 4 meters	5192700
*sens**ion*™ 2 meters	5192700

Before starting the test:

Refer to the meter manual for meter operation. Refer to electrode manual for electrode maintenance and care.
Prepare the electrode. Refer to *New electrodes or electrodes stored more than 7 days* and *Electrodes stored 1 to 7 days* for more information.
After every hour of continuous use, place the electrode in the storage solution for 10 minutes to thoroughly recondition. Check with a 10 mg/L NH_3–N standard for accuracy and calibrate if necessary.
At high pH, ammonia solutions lose ammonia to the atmosphere, lowering the concentration. It is important to take measurements as soon as possible after the solution is basic. For most wastewater samples, 1 mL of 10 N NaOH (or equivalent ISA) is sufficient to increase the pH above 11. If in doubt, check the pH with pH paper and add additional NaOH in 0.1 mL increments until the pH exceeds 11.

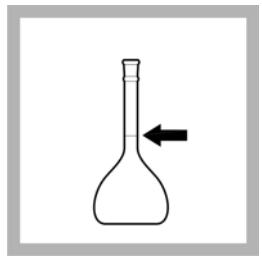
Collect the following items:

Description	Quantity
Ammonia electrode filling solution	varies
Ammonia electrode storage solution	20 mL
Ammonia nitrogen standard, 100 mg/L NH_3–N	varies
Water, deionized	100 mL
Ammonia ISA solution	2 mL per 100 mL sample
Ammonia electrode, combination BNC	1
Beaker, 150 mL, polypropylene	4
Wash bottle	1
TenSette® pipet, 1.0–10.0 mL	1
*sens**ion*** 4 laboratory pH/ISE meter or sension 2 portable pH/SE meter	1
Stir bar, 22.2 x 4.76 mm (7/16 x 3/16 in.)	4
Stirrer, electromagnetic, with stand and stir bar	1
25 mL Class A volumetric pipet	1
250 mL Class A volumetric flask	3

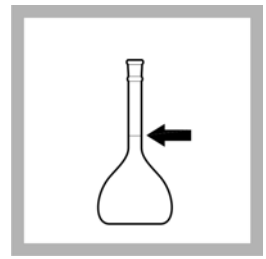
See *Consumables and replacement items* for reorder information.

Nitrogen, Ammonia in wastewater method

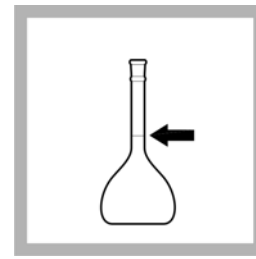
1. Rinse the electrode with deionized water. Place it in the Ammonia ISE storage solution with the ammonia membrane sensor module on to condition for at least 15 minutes.



2. During the conditioning period prepare three standards. Make a 10-mg/L NH_3–N standard by pipeting 25 mL of 100-mg/L NH_3–N Standard into a 250-mL volumetric flask. Dilute to the mark with ammonia -free deionized water, stopper and thoroughly mix.



3. Prepare a 1.0-mg/L NH_3–N standard by pipeting 25 mL of the 10-mg/L standard into a 250-mL volumetric flask. Dilute to the mark with ammonia-free deionized water, stopper and thoroughly mix.

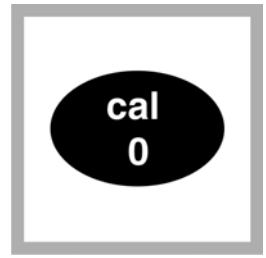


4. Prepare a 0.1-mg/L NH_3–N standard by pipeting 25 mL of the 1.0-mg/L NH_3–N standard into a 250-mL volumetric flask. Dilute to the mark with ammonia -free deionized water, stopper and thoroughly mix.

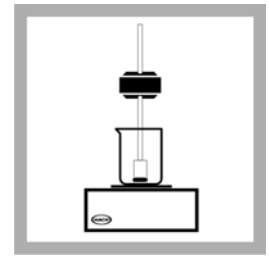
Nitrogen, Ammonia in wastewater method (continued)

5. Connect the Ammonia ISE to the BNC connector on the pH/ISE meter. Verify that BNC is selected in Setup 1 of the Setup menu.

6. Turn the meter on. Press **ISE/MV** until the display shows mg/L or other chosen concentration units.



7. Press **CAL**. Use the **ARROW** keys to select the desired units. Press **ENTER** and accept the units.



8. Transfer 100 mL of the 0.1-mg/L NH_3–N standard to a 150-mL beaker. Add a stir bar to the beaker. Put the beaker on a magnetic stirrer and stir at a moderate rate.

9. Remove the electrode from the storage solution. Rinse it with deionized water and blot dry. Put the electrode into the 0.1-mg/L NH_3–N standard.

Make sure no air bubbles are trapped under the tip of the electrode.

10. Pipet 2.0 mL of Ammonia ISA Solution into the standard. Immediately proceed to the next step.

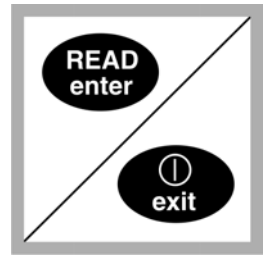
11. Press **READ.** The display will show the value from the previous calibration. Accept the numerical value or use the number keys to change the value to match the concentration of the standard, then press **ENTER** to accept the change.

12. The display will show **Stabilizing...** until the reading is stable. The display will show **Standard 2** and _ _ _ _ or the value of standard 2 from the previous calibration.

Nitrogen, Ammonia in wastewater method (continued)

13. Rinse the electrode with deionized water. Place it in the storage solution for one minute. Repeat steps 8–13 for substituting the 1.0- and 10-mg/L standards.

14. After the last standard is measured, press **EXIT**.



15. The display will show **Store?**. Press **ENTER** to store the calibration or **EXIT** to leave the calibration mode without storing the calibration values.

16. Press **REVIEW**. Use the Up arrow key to scroll to the second slope value. It should be –57 ± 3 mV/decade. If the slope is not –57 ± 3 mV/decade, recalibrate the electrode. If the slope is still incorrect after recalibration, replace the ammonia membrane sensor module.

Press **EXIT** to return to measurement mode.

17. Remove the electrode from the last standard. Rinse it with deionized water and place it in the storage solution.

18. Transfer 100 mL of sample to a 150-mL beaker. Add a stir bar to the beaker. Put the beaker on a magnetic stirrer and stir at a moderate rate.

19. Remove the electrode from the storage solution. Rinse with deionized water and blot dry. Put the electrode into the sample.

20. Pipet 2.0 mL of Ammonia ISA solution into the sample and proceed immediately to the next step.

Nitrogen, Ammonia in wastewater method (continued)

21. Press **READ**. The display will show Stabilizing... until the reading is stable. Record or store the measurement value.

Repeat steps 17–21 for other samples.

Stabilization times will take longer for lower concentrations. A slow downward drift in concentration indicates probable loss of ammonia to the atmosphere. Record the highest value that is stable.

Calibration

Prepare ammonia standard working solutions of 10.0, 1.0 and 0.1 mg/L ammonia nitrogen from a 100-mg/L stock solution. Prepare the standards daily before use. Higher or lower concentration ranges (0.05–1400 mg/L NH_3–N) can be obtained by calibrating the meter with different standard solutions.

Electrode preparation

New electrodes or electrodes stored more than 7 days

Before using a new Ammonia Electrode or an electrode that has been stored dry, remove the protective cap from the end.

1. Unscrew the top cap. Carefully remove the internal glass electrode from the outer body. A white membrane is mounted at the tip of the outer body.
2. Fill the outer body with 3.5 mL of Internal Fill Solution.
3. Rinse the internal glass electrode with deionoized water. Blot dry. Return the electrode to the filled outer body. Make sure that the key pin at the top of the internal glass electrode is seated in the slot at the top of the outer body.
4. Reinstall the threaded top cap onto the top of the ammonia electrode body. Finger-tighten the cap until snug. **Do not over-tighten.**

5. Hold the fully assembled electrode securely by one end and shake the electrode with an abrupt downward motion (like shaking the mercury down in a thermometer) to remove bubbles.

6. Place the assembled electrode into the Ammonia Electrode Storage Solution or 1000 mg/L Ammonia Standard for at least 60 minutes.

Electrodes stored 1 to 7 days

- Keep the electrode in 1000 mg/L ammonia standard without Ionic Strength Adjustor (ISA).
- Alternatively, keep the electrode in the Ammonia Electrode Storage Solution.
- Never let the membrane dry out. Cover the storage beaker and electrode body with Parafilm® to prevent solution evaporation.

Electrodes stored between samples

Place the electrode in Ammonia Electrode Storage Solution for at least one minute to initialize the electrode for the next measurement.

Interferences

Distillation prior to ammonia analysis removes all inorganic interferences that complex ammonia.

Table 408 Interfering substances

Interfering substance	Interference level
Amines	Volatile low molecular weight gives a positive interference
Mercury	Complexes with ammonia
Silver	Complexes with ammonia

Sample collection, preservation and storage

- Collect samples in glass or plastic containers of convenient size. Clean new bottles by washing with deionized or distilled water. Fill the sample bottle completely and stopper immediately. Analyze the sample as soon as possible.
- Ammonia may be lost more quickly from samples at temperatures above 50 °C, so it is important to collect samples at less than 40 °C or use a cooling coil between the bottle and sampling point if necessary.
- If chlorine is present, treat the sample immediately with sodium thiosulfate. Add one drop of 0.1 N Sodium Thiosulfate Standard Solution for each 0.3 mg of chlorine present in a one liter sample.
- If prompt analysis is not possible, preserve the sample with 0.8 mL of concentrated sulfuric acid per liter. Use a sension pH meter to be sure the pH of the preserved sample is between 1.5 and 2. Some wastewater samples may require more sulfuric acid to achieve this pH. Store the sample at 4 °C. Samples preserved in this manner may be stored up to 28 days.
- Before analysis, neutralize the sample to pH 7 with 5 N sodium hydroxide. Do not let the pH go above 10. Correct the test results for the volume addition.
- Do not use mercuric chloride as a preservative because ammonia complexes with mercuric ions.

Accuracy check

Standard additions method (sample spike)
To verify measurement accuracy, perform a standard addition spike on the sample. The spike should roughly double the measured concentration without significantly diluting the sample.

To perform a standard addition sample:

1. Use the *Spike volumes for standard additions* table to determine the concentration and volume of standard to spike the sample. The volume of sample transferred must be accurate.
2. Add the amount and concentration specified in the *Spike volumes for standard additions* table to the 100 mL of sample.
3. After adding the standard, proceed with the calculations. Results from 90-110% recovery are typically considered acceptable. Calculate percent recovery as follows:

$$\% \text{ Recovery} = \frac{100(X_s - X_u)}{K}$$

Where:
X_s = measured value for spiked sample in mg/L

X_u = measured value for unspiked sample adjusted for dilution by the spike, in mg/L

K = known value of the spike in the sample in mg/L

Calculations

1. $X_u = \frac{X_i \times V_u}{V_u + V}$

Where:

X_i = measured value of unspiked sample in mg/L

V_u = volume of separate unspiked portion in mL

V = volume of spike in mL

2. $K = \frac{C \times V}{V_u + V}$

Where:

C = concentration of standard used in spike in mg/L

V = volume of spike in mL

V_u = volume of separate portion before spike in mL

3. Final calculation plugging in X_u and K: $\% \text{ Recovery} = \frac{100(X_s - X_u)}{K}$

Example:

A sample was analyzed and read 5.0 mg/L NH_3–N. As directed in the *Spike volumes for standard additions* table, a 4.0-mL spike of 100-mg/L NH_3–N standard was added to another 100-mL sample, giving a final standard addition result of 8.75 mg/L.

Calculate the percent recovery as follows:

1. $X_u = \frac{5.0\ mg/L \times 100\ mL}{100\ mL + 4\ mL} = 4.81\ mg/L$

2. $K = \frac{100\ mg/L \times 4\ mL}{100\ mL + 4\ mL} = 3.85\ mg/L$

3. $\%R = \frac{100 \times (X_s - X_u)}{K} = \frac{100 \times (8.75 - 4.81)}{3.85} = 102.3\ \%\ Recovery$

Table 409 Spike volumes for standard additions

Measured Sample Concentration (mg/L)	Measured Sample Volume (mL)	Standard Concentration (mg/L)	Standard Volume (mL)
0.1–0.3	100	100	0.2
0.3–0.5	100	100	0.4
0.5–0.7	100	100	0.6
0.7–0.9	100	100	0.8
0.9–1.1	100	100	1.0
1.0–3.0	100	100	2.0
3.0–6.0	100	100	4.0
6.0–10.0	100	100	8.0

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
*sens**ion*** 4[1]	0.80 mg/L	0.78–0.82 mg/L
*sens**ion*** 2[1]		

[1] With a default stabilization criteria of 0.5 mV/min.

Summary of method

The ammonia electrode measures ammonia gas or ammonium ions in aqueous solutions that have been converted to gas by the addition of a strong base. The electrode is a complete electrochemical cell consisting of a glass pH electrode and a reference electrode.

The gas-permeable membrane separates the sample from a thin layer of electrolyte that is pressed between the pH bulb and the membrane. At high pH, ammonium ion is converted to ammonia gas.

The gas diffuses through the membrane and causes a pH change in the thin layer of electrolyte. The potential across the pH glass changes as a result of the pH change and the electrode measures the change in potential. The measured pH change is proportional to the ammonia concentration in the solution.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode Filling Solution	varies	60 mL	4447226
Ammonia Electrode Storage Solution	20 mL	500 mL	2541249
Ammonia Nitrogen Standard, 100 mg/L NH_3-N	100 mL	500 mL	2406549
Ammonia ISA Solution	2 mL/100 mL sample	500 mL	2824349
Water, deionized	100 mL	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode	1	each	5192700
Beaker, 150 mL, polypropylene	4	each	108044
Bottle, wash, 500 mL	1	each	62011
Flask, volumetric, Class A, 250 mL	3	each	1457446
*sens**ion*** 4 Laboratory pH/ISE Meter or *sens**ion*** 2 pH/ISE (portable)	1	each	5177500
Stir Bar, 22.2 x 4.76 mm	4	each	4531500
TenSette® Pipet, 1.0–10.0 mL	1	each	1970010
Pipet tips for 1970010 TenSette Pipet	varies	50/pkg	2199796
Class A 25 mL volumetric pipet	1	each	1451540
Safety bulb pipet filler	1	each	1418900
Select one based on available voltage:			
Stirrer, electromagnetic 115 V, with stand and stir bar	1	each	4530001
Stirrer, electromagnetic 230 V, with stand and stir bar	1	each	4530002

Optional reagents

Description	Unit	Catalog number
Ammonia Nitrogen Standard Solution 1000 mg/L NH_3-N	1 L	2354153
pH Paper, pH 9.0-12.0	5 rolls/pkg	38533
Sulfuric Acid, concentrated	500 mL	97949

Optional apparatus

Description	Unit	Catalog number
Air Gap Assembly	each	5025300
Ammonia Electrode Membrane Modules	4/pkg	5192711
Cylinder, graduated, glass	100 mL	50842
Electrode Washer	each	2704700
Pipet, Volumetric, Class A, 1.00 mL	each	1451535
TenSette® Pipet, 0.1–1.0 mL	each	1970001
Pipet tips for 1970001 TenSette Pipet	50/pkg	2185696
*sens**ion*** 2 Portable pH/ISE Meter	each	5172500

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Edition 7

Nitrogen, Ammonia

DOC316.53.01234

USEPA[1] Known Addition ISE Method[2] **Method 10002**

≥0.8 mg/L NH_3-N **ISE Electrode**

Scope and Application: For wastewater

[1] USEPA Accepted for reporting wastewater analyses

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, Method 4500NH3E (with distillation). Manual distillation is not required if comparability data on representative samples in company files show the distillation is not necessary. Manual distillation will be required to resolve any controversies.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 410 Instrument-specific information

Meter	Electrode
*sens**ion***™ 4 meters	5192700

Before starting the test:

Refer to the meter manual for meter operation. Refer to electrode manual for electrode maintenance and care.
Prepare the electrode:
New electrodes or electrodes stored more than 7 days
Electrodes stored 1 to 7 days
Refer to the Distillation Apparatus manual to perform sample distillation, if necessary.
Prepare spiking solutions according to the Nitrogen and Ammonia in the Wastewater section.
When there is a linear relationship between concentration and response, the known addition method can be used to measure occasional samples because calibration is not required. The sample concentration must be known within a factor of three, in order to get accurate measurements, because the concentration of ammonia in the sample must be approximately doubled by the standard addition.

Collect the following items:

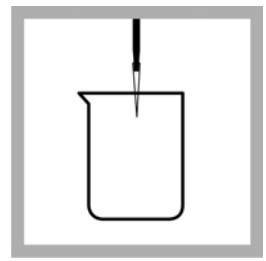
Description	Quantity
Ammonia electrode filling solution	varies
Ammonia electrode storage solution	20 mL
Ammonia nitrogen standard, 1000 mg/L NH_3–N	varies
Ammonia ISA solution	2 mL per 100 mL sample
Ammonia electrode, combination BNC	1
Wash bottle	1
TenSette® pipet, 1.0–10.0 mL	1
*sens**ion*** 4 laboratory pH/ISE meter	1
Stir bar, 22.2 x 4.76 mm (7/16 x 3/16 in.)	1
Select on based on available voltage:	
Stirrer, electromagnetic, 115 V, with stand and stir bar	1
Stirrer, electromagnetic, 230 V, with stand and stir bar	1

See *Consumables and replacement items* for reorder information.

Nitrogen, Ammonia known addition method

1. Accurately transfer 100 mL of sample to a 150-mL beaker using a volumetric pipet or graduated cylinder. Add a stir bar to the beaker.

2. Stir at a constant and moderate rate on a magnetic stirrer to improve response time and accuracy.



3. Use a TenSette Pipet to add 1.0 mL of 10 N NaOH solution into the sample.

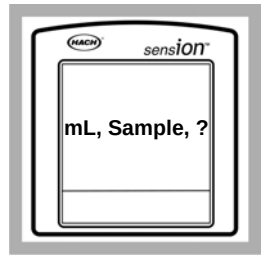
4. Remove the electrode from the storage solution. Rinse it with deionized water and blot dry. Put the electrode in the sample.

Make sure that no air bubbles are trapped under the tip of the electrode. Remove bubbles by lightly tapping the electrode or by tilting the electrode to 20°.

Nitrogen, Ammonia known addition method (continued)

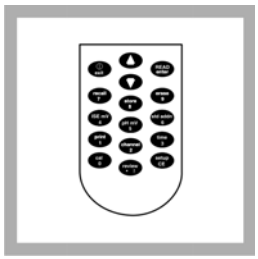
5. Turn the meter on. Press **STD ADDN**. Use the **ARROW** keys to select the required units. Accept the units.

6. The display will show the slope value for the last calibration (the default is –59.2 mV). Accept the numerical value or change the slope value.



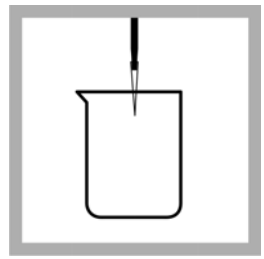
7. The meter will prompt for the sample volume (in mL). Enter the sample volume and press **ENTER** to accept the volume.

8. The display will show **Stabilizing...** until the baseline reading is stable. The meter will then prompt for the standard volume.

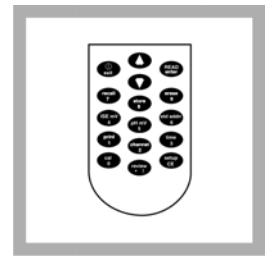


9. Enter the volume of standard to be used (for example, 1.0 mL). Press **ENTER** and accept the volume.

10. Obtain the standard concentration and volume from Table 4 on page 48 after estimating the sample concentration.



11. Add the volume of a known standard (listed in Table 4) to the beaker and proceed as quickly as possible through the rest of the procedure.



12. Enter the concentration of the standard used (for example, 1000 mg/L).

Press **ENTER** and accept the concentration.

Nitrogen, Ammonia known addition method (continued)

13. The display will show **Sample+Standard** and **Stabilizing...** until the reading is stable.

14. The meter will calculate and display the concentration of the adjusted value for the original sample in mg/L. Record or store this value.

The display will show **STANDARD ADDITONS** when data is recalled for standard additions.

Nitrogen ammonia in wastewater

Known addition is also a convenient check on the results of direct measurement. Because an accurate measurement requires that the concentration approximately double as a result of the addition, the approximate sample concentration must be known within a factor of three. Make a spiking solution before beginning the procedure.

1. Use the *Spiking solutions* table to determine how to dilute a 1000 mg/L NH_3-N stock solution to use as a spiking solution.
2. Pipet the appropriate amount of 1000-mg/L NH_3-N standard into a 100-mL volumetric flask.
3. Dilute to the mark with ammonia-free water.
4. Determine the slope before performing standard additions of the sample.
   a. Use the 100 mg/L and 1000-mg/L NH_3-N stock solutions to determine the slope.
   b. Check the electrode occasionally to determine if it is functioning properly and to determine its exact slope value. The frequency of this operation depends on the harshness of the sample.

Table 411 Spiking solutions

Expected Sample Concentration (mg/L)	mL of 1000-mg/L NH_3-N	Standard Concentration
0.8–4.0	2	20 mg/L
2.5–7.5	5	50 mg/L
5–15	10	100 mg/L
12–50	25	250 mg/L
25–75	50	500 mg/L
50–150	100	1000 mg/L

Electrode preparation

New electrodes or electrodes stored more than 7 days

Before using a new Ammonia Electrode or an electrode that has been stored dry, remove the protective cap from the end.

1. Unscrew the top cap. Carefully remove the internal glass electrode from the outer body. A white membrane is mounted at the tip of the outer body.
2. Fill the outer body with 3.5 mL of Internal Fill Solution.
3. Rinse the internal glass electrode with deionoized water. Blot dry. Return the electrode to the filled outer body. Make sure that the key pin at the top of the internal glass electrode is seated in the slot at the top of the outer body.
4. Reinstall the threaded top cap onto the top of the ammonia electrode body. Finger-tighten the cap until snug. **Do not over-tighten.**
5. Hold the fully assembled electrode securely by one end and shake with an abrupt downward motion (like shaking the mercury down in a thermometer) to remove bubbles.
6. Place the assembled electrode into the Ammonia Electrode Storage Solution or 1000 mg/L Ammonia Standard for at least 60 minutes.

Electrodes stored 1 to 7 days

- Keep the electrode in 1000 mg/L ammonia standard without Ionic Strength Adjustor (ISA).
- Alternatively, keep the electrode in the Ammonia Electrode Storage Solution.
- Never let the membrane dry out. Cover the storage beaker and electrode body with Parafilm® to prevent solution evaporation.

Electrodes stored between samples

Place the electrode in Ammonia Electrode Storage Solution for at least one minute. This reinitializes the electrode for the next measurement.

Interferences

Distillation prior to ammonia analysis removes all inorganic interferences that complex ammonia.

Table 412 Interfering substances

Interfering substance	Interference level
Amines	Volatile low molecular weight gives a positive interference
Mercury	Complexes with ammonia
Silver	Complexes with ammonia

Sample collection, preservation and storage

- Collect samples in glass or plastic containers of convenient size. Clean new bottles by washing with deionized or distilled water. Fill the sample bottle completely and stopper immediately. Analyze the sample as soon as possible.
- Ammonia may be lost more quickly from samples at temperatures above 50 °C, so it is important to collect samples at less than 40 °C or use a cooling coil between the bottle and sampling point if necessary.
- If chlorine is present, treat the sample immediately with sodium thiosulfate. Add one drop of 0.1 N Sodium Thiosulfate Standard Solution for each 0.3 mg of chlorine present in a one liter sample.

- If prompt analysis is not possible, preserve the sample with 0.8 mL of concentrated sulfuric acid per liter. Use a sension pH meter to be sure the pH of the preserved sample is between 1.5 and 2. Some wastewater samples may require more sulfuric acid to achieve this pH. Store the sample at 4 °C. Samples preserved in this manner may be stored up to 28 days.
- Before analysis, neutralize the sample to pH 7 with 5 N sodium hydroxide. Do not let the pH go above 10. Correct the test results for the volume addition.
- Do not use mercuric chloride as a preservative because ammonia complexes with mercuric ions.

Accuracy check

Standard additions method (sample spike)
To verify measurement accuracy, perform a standard addition spike on the sample. The spike should roughly double the measured concentration without significantly diluting the sample.

To perform a standard addition sample:

1. Use the *Spike volumes for known additions* table to determine the concentration and volume of standard to spike the sample. The volume of sample transferred must be accurate.
2. Add the amount and concentration specified in the *Spike volumes for known additions* table to the sample while performing the standard addition method on the sample. Do not allow the sample to stand too long before spiking or ammonia will be lost to the atmosphere. T
3. After adding the standard, proceed with the calculations. Results from 90–110% recovery are typically considered acceptable. Calculate percent recovery as follows:

$$\% \text{ Recovery} = \frac{100(X_s - X_u)}{K}$$

Where:

X_s = measured value for spiked sample in mg/L

X_u = measured value for unspiked sample adjusted for dilution by the spike, in mg/L

K = known value of the spike in the sample in mg/L

Calculations

1. $X_u = \frac{X_i \times V_u}{V_u + V}$

Where:

X_i = measured value of unspiked sample in mg/L

V_u = volume of separate unspiked portion in mL

V = volume of spike in mL

2. $K = \frac{C \times V}{V_u + V}$

Where:

C = concentration of standard used in spike in mg/L

V = volume of spike in mL

V_u = volume of separate portion before spike in mL

3. Final calculation plugging in X_u and K: $\% \text{ Recovery} = \frac{100(X_s - X_u)}{K}$

Example:

A sample was analyzed and read 5.0 mg/L NH_3–N. As directed in the *Spike volumes for known additions* table, a 4.0-mL spike of 100-mg/L NH_3–N standard was added to another 100-mL sample, giving a final standard addition result of 8.75 mg/L.

Calculate the percent recovery as follows:

1. $X_u = \frac{5.0\ mg/L \times 100\ mL}{100\ mL + 4\ mL} = 4.81\ mg/L$

2. $K = \frac{100\ mg/L \times 4\ mL}{100\ mL + 4\ mL} = 3.85\ mg/L$

3. $\%R = \frac{100 \times (X_s - X_u)}{K} = \frac{100 \times (8.75 - 4.81)}{3.85} = 102.3\ \%\ Recovery$

Table 413 Spike volumes for known additions

Measured Sample Concentration (mg/L)	Measured Sample Volume (mL)	Standard Concentration (mg/L)	Standard Volume (mL)
0.8–1.0	100	100	1.0
1–3	100	100	2.0
3–6	100	100	4.0
6–9	100	100	8.0
9–12	100	100	10.0
12–20	100	1000	2.0
20–40	100	1000	4.0
40–60	100	1000	6.0
60–75	100	1000	8.0

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
*sens**ion*** 4	5.00 mg/L	4.81–5.19 mg/L
*sens**ion*** 2		4.81–5.19 mg/L

Summary of method

The ammonia electrode measures ammonia gas or ammonium ions in aqueous solutions that have been converted to gas by the addition of a strong base. The electrode is a complete electrochemical cell consisting of a glass pH electrode and a reference electrode.

The gas-permeable membrane separates the sample from a thin layer of electrolyte that is pressed between the pH bulb and the membrane. At high pH, ammonium ion is converted to ammonia gas.

The gas diffuses through the membrane and causes a pH change in the thin layer of electrolyte. The potential across the pH glass changes as a result of the pH change and the electrode measures the change in potential. The measured pH change is proportional to the ammonia concentration in the solution.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode Filling Solution	3 mL	50 mL	4447226
Ammonia Electrode Storage Solution	5 mL	500 mL	2541249
Ammonia Nitrogen Standard, 1000 mg/L NH_3-N	varies	1 L	2354153
Sodium Hydroxide Solution, 10 N	10 mL	500 mL	2545049
Water, deionized	100 mL	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode	1	each	5192700
Beaker, 150 mL, polypropylene	1	each	108044
Bottle, wash, 500 mL	1	each	62011
Cylinder, graduated, 100-mL	1	each	50842
*sens**ion*** 4 Laboratory pH/ISE Meter	1	each	5177500
Stir Bar, 22.2 x 4.76 mm	1	each	4531500
TenSette® Pipet, 1.0–10.0 mL	1	each	1970010
Pipet tips for 1970010 TenSette Pipet	varies	50/pkg	2199796
Select one based on available voltage:			
Stirrer, electromagnetic 115 V, with stand and stir bar	1	each	4530001
Stirrer, electromagnetic 230 V, with stand and stir bar	1	each	4530002

Optional reagents

Description	Unit	Catalog number
pH Paper, pH 9.0-12.0	5 rolls/pkg	38533
Sulfuric Acid, concentrated	500 mL	97949
5.0 N NaOH	1000 mL	245053

Optional apparatus

Description	Unit	Catalog number
Ammonia Electrode Membrane Modules	4/pkg	5192711
Electrode Washer	each	2704700
Pipet, Volumetric, Class A, 1.00 mL	each	1451535
Pipet, Volumetric, Class A, 10.00 mL	each	1451538
Pipet, Volumetric, Class A, 100.0 mL	each	1451542
Pipet tips for 1970001 TenSette Pipet	50/pkg	2185696

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Edition 7

Sodium

DOC316.53.01240

Direct Measurement ISE Method **Method 8359**

10 to 2000 µg/L Na^+ **ISE Electrode**

Scope and Application: Drinking water and process water application

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 414 Instrument-specific information

Meter	Electrode
*sens**ion***™ 4 meters[1]	5192000

[1] Only *sens**ion*** 4 meters can be used for this analysis.

Before starting the test:

Before the test, prepare a 10 mg/L Na^+ standard:

1. Use a Class A pipet to dispense 10 mL of 1000 mg/L Na^+ standard into a 1.0 L volumetric flask.
2. Dilute to the mark with deionized water.

This procedure requires several conditioning steps. Read through all the procedure steps before proceeding.

This test is for low range analysis only. If the electrode is conditioned to a low sodium concentration (as in this procedure), putting it in a concentrated Na^+ (10 mg/L or higher) will swamp the electrode with sodium ion and the overnight conditioning step must be repeated.

Measure millivolt potentials of all sodium standards at the same temperature ± 0.5 °C. Samples and standards must be measured at the same temperature, ± 1 °C. Use room temperature 100 mg/L Na^+ standard. This procedure keeps temperature error to a minimum by using a spiked additions method of calibration.

Note A: If the electrode is conditioned properly, the mV potential should not change more than 0.1 mV every minute in step 10. If excessive drift occurs leave the electrode in the 600-mL beaker until the drift slows. If the electrode drifts 10 mV or more in the positive direction (for example, from –175 to –165 mV), repeat steps 5–10, but do not dispense electrolyte gel again as directed in step 7.

Collect the following items:

Description	Quantity
Potassium chloride electrolyte gel cartridge	1
pH/ISE meter, *sens**ion***™4 (laboratory)	1
Sodium ISE Electrode, BNC	1
Sodium ISA powder pillows	1

Collect the following items: (continued)

Description	Quantity
Sodium standard solutions:	
100 mg/L Na^+	
1000 mg/L Na^+	
Water, deionized	varies
Flask, 100-mL, volumetric	1
Beaker, 600-mL, polypropylene	2
Bottle, wash, 500-mL	1
Cylinder, graduated, 500-mL, polypropylene	3
Pipet, TenSette™, 0.1 to 1.0 mL, plus tips	1
Stir Bar, $^7/_8$ X $^3/_{16}$ in. (22.2 x 4.8 cm)	1
Stirrer, electromagnetic with stand and stir bar	1

See *Consumables and replacement items* for reorder information.

Sodium ISE, powder pillow method

1. Install the Potassium Chloride Electrolyte Gel Cartridge in the Platinum Series Combination Sodium Electrode.

2. Connect the combination sodium electrode to the meter.

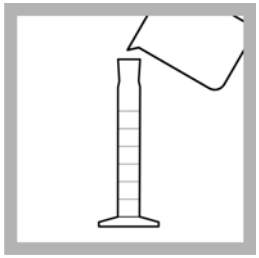
Ensure the electrode has been conditioned for at least 8 hours in Sodium Electrode Storage Solution before its initial use.

3. Prime the electrode by pushing the dispenser button until gel comes out of the reference junction. Rinse excess gel from the tip and the outlet.

4. Condition the electrode in sodium electrode storage solution for a minimum of 1 hour before use. Then, condition the electrode in 0.10 mg/L sodium for at least 8 hours.

To make 100 mL of 0.10 mg/L Na+ standard, use a TenSette™ Pipet to put 0.10 mL of 100 mg/L Na+ into a 100-mL volumetric flask and dilute to the mark. Mix well.

Sodium ISE, powder pillow method (continued)



5. Accurately measure 400 mL of deionized water into a plastic 500-mL graduated cylinder.

The deionized water must be at room temperature.

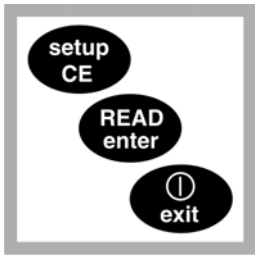
6. Pour the water in the cylinder into a 600-mL plastic beaker. Add the contents of one Sodium Ionic Strength Adjustor powder pillow to the solution.

Add a large stir bar (50.8 x 7.9 mm) to the beaker. Place the beaker on an electromagnetic stirrer and begin stirring at a moderate rate.

7. Remove the electrode from the 0.10 mg/L sodium standard, dispense gel and rinse excess gel away with deionized water. Place the electrode in the 600-mL beaker, submerging the tip below the solution surface.

8. Use a TenSette® Pipet to add 0.4 mL of 10 mg/L Sodium Standard Solution to the solution in the beaker. Refer to the *Low level sodium calibration* table. (This makes 400 mL of 0.010 mg/L or 10 µg/L sodium.)

Allow the electrode to condition for 15 minutes in this solution before proceeding.



9. Turn the meter on. Set the electrode type to BNC.

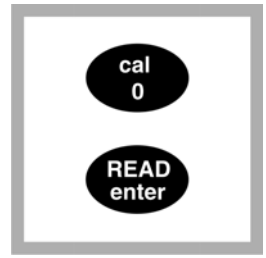
Press **SETUP** and scroll to Stabilizing.

Press **ENTER** and edit the display to show 0.1 mg/min. Accept the value and **EXIT** the setup menu.

10. Press **ISE MV** until the mV potential shows on the display. Refer to *Before starting the test: Note A*.

After the measurement is stable, record the potential value.

Measure the temperature of the standard (°C) with a lab grade thermometer. Record the temperature.



11. Press **ISE MV** to toggle to concentration units. Press **CAL** and scroll to µg/L. Press **ENTER** to accept the concentration units.

12. Edit the display to show the concentration of the solution in the 600 mL beaker (10 µg/L). Refer to the *Low level sodium calibration* table.

Press **ENTER** to accept the concentration.

Sodium ISE, powder pillow method (continued)

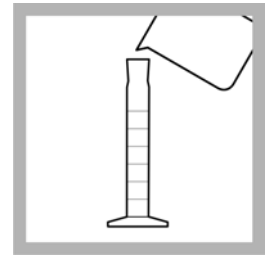
13. When the measurement stabilizes, pipet the corresponding additional volume from the 10-mg/L Na+ standard in the *Low level sodium calibration* table. Wait the amount of time specified for step 2 in the table to allow the membrane to respond.

Enter the concentration in µg/L (20 µg/L). Press **ENTER** to accept the concentration.

14. Repeat step 13, adding the additional volumes of 10 mg/L and 100 mg/L Na^+ standard from the *Low level sodium calibration* table until all seven standards have been measured. Store the calibration and return to measurement mode.

15. Remove the electrode from the last standard, rinse well with deionized water and blot dry.

Save the solution in the 600-mL beaker with the 2.00 mg/L Na^+ for later calibration checks.



16. Accurately measure 400 mL of sample into a 500-mL graduated cylinder.

The sample must be at the same temperature as the standard solution in the 600-mL beaker used to perform the calibration.

Sodium ISE, powder pillow method (continued)

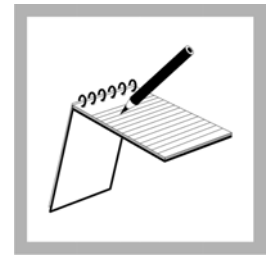
17. Pour this 400 mL of sample into a 600-mL beaker. Add a magnetic stir bar, place the beaker on an electromagnetic stirrer. Stir at a moderate rate.

If the electrode is placed in a sample of high sodium concentration, errors may result and reconditioning to lower sodium levels will be required.

18. Add the contents of one Sodium Ionic Strength Adjustor Powder Pillow.

19. Place the electrode into the sample.

Wait 10 minutes to allow electrode to condition to the low level sodium in solution.



20. When the measurement stabilizes, record the sample concentration value.

If the sodium concentration is more than the 10 to 2000 g/L calibration range, press the ISE mV key. If the mV reading is greater than the mV at 2000 g/L Na+, analyze the sample using the procedure for Potable, Ground and Irrigation Water (refer to the Sodium electrode manual). If the mV reading is less than the mV reading of the 10 g/L standard, there is less than 10 g/L Na+ in the sample.

Calibration

Use a water bath slightly above room temperature (25 °C) to equilibrate the standard temperature and sample temperature before measuring mV potentials. Use a laboratory-grade thermometer to monitor the temperature. A one degree centigrade difference may result in as much as a 0.4 mV inaccuracy. This temperature variation will, in turn, decrease accuracy of concentration measurements.

Table 415 Low level sodium calibration

Step	Volume 10 mg/L Na^+ standard added	Volume 100 mg/L Na^+ standard added	Concentration	
			µg/L	Time
1	0.4 mL		10	until mV stabilizes
2	0.4 mL		20	15 min.
3	1.2 mL		50	15 min.

Table 415 Low level sodium calibration

Step	Volume 10 mg/L Na^+ standard added	Volume 100 mg/L Na^+ standard added	Concentration	
			µg/L	Time
4		0.2 mL	100	10 min.
5		0.4 mL	200	10 min.
6		1.2 mL	495	10 min.
7		2.0 mL	1000	10 min.
8		4.0 mL	2000	10 min.

Interferences

The Sodium ISA is formulated to remove most interferences. Silver is a major interference.

Sample collection, preservation and storage

Analyze immediately after sampling. If immediate analysis is impossible, cool samples to 4 °C and analyze within six hours

Low ionic strength conditioning

The conditioning steps in the section apply to measurements in samples containing less than 1.0 mg/L Na^+.

Initial use

The sensing half-cell is packaged and shipped dry from the factory with a dry cap over the glass membrane tip.

1. Remove the cap and install the gel cartridge.
2. Click the reference electrolyte dispenser until gel emerges from the tip.
3. Rinse the electrode with a small stream of sample delivered through a disposable plastic Pasteur pipette or with deionized water from a wash bottle.
4. Place the tip in Sodium Electrode Storage Solution or in 1 M sodium chloride solution and soak for at least one hour. Condition the electrode in 0.10 mg/L sodium solution for at least 8 hours before calibrating using the Low Range Sodium Method. Refer to the electrode user manual for more information.

Between uses

Between uses, in intervals of up to a few hours, the electrode can be stored in the sample (if not an extreme pH) or in a neutral low-ionic-strength solution such as tap water. Before measuring a new sample, refresh the reference electrolyte gel by clicking the dispenser several times. Carefully rinse the electrode to prevent contaminating the sample.

Accuracy check

Electrode response

To verify electrode response at these low levels of sodium, the millivolt potential should increase upon each addition of 100 mg/L Na^+. Using the Low Level Sodium procedure, at least a 4.0 mV increase should be observed from step 1 to step 2 (10 µg/L to 20 µg/L Na^+). Each additional spike should increase the mV reading substantially from the previous change. If this is not the case, check the purity of the standard used. If this is not the problem, the electrode is probably not conditioned for low sodium levels.

Calibration accuracy

1. Fill the 500-mL graduated cylinder to the 400-mL mark with deionized water.
2. Pour this solution in a 600-mL beaker and add a stir bar.
3. Pipet 0.2 mL of 100 mg/L Sodium Standard into the 600-mL beaker.
4. Add the contents of one Sodium Ionic Strength Adjustor powder pillow and place on an electromagnetic stirrer.
5. Rinse the calibrated electrode before placing in solution. Measure the concentration of the solution. The reading should be approximately 50 µg/L.

Note: *The beaker with the 2000 µg/L Na+ standard used in the calibration may be used as a check on the calibration. It should read close to 2000 µg/L.*

Accuracy of the sample measurement

To verify sample measurement accuracy, add a spike of standard solution with a TenSette® or volumetric pipet. Use the *Spike volumes of 100-mg/L standard* table and the formulas in *Percent recovery*.

Table 416 Spike volumes of 100-mg/L standard

Measured sample Concentration Na^+ (µg/L)	Volume of 100 ppm Standard to Add	C x V
10 to 49	0.1 mL	10
50 to 99	0.2 mL	20
100 to 299	0.4 mL	40
300 to 599	1.2 mL	120
600 to 2000	2.0 mL	200

Percent recovery

To calculate the percent recovery:

$$M = S \times 400 + (C \times V)$$

$$E = \left[\frac{M}{400 + V}\right]$$

$$R = \frac{A}{E} \times 100\%$$

Where:

M = calculated mass of sodium present after the spike (micrograms)
S = mg/L of Na^+ in sample (before spike)
C = concentration of standard used for spiking (mg/L)
V = spike volume from the *Spike volumes of 100-mg/L standard* table (mL)
E = expected concentration after spiking (mg/L)
R = percent recovery (should be 95–100%)
A = actual reading on meter after spike

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
*sens**ion*** 4[1]	25 µg/L	21.34–28.66 µg/L

[1] With a stabilization criteria of 0.1 mV/min

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Potassium Chloride Reference Electrolyte Gel Cartridge	varies	3/pkg	.2546902
Sodium Ionic Strength Adjustor Powder Pillows	varies	100/pkg	4451569
Sodium Standard Solutions 100 mg/L as Na^+	varies	1000 mL	2318153
Sodium Standard Solutions 1000 mg/L as Na^+	varies	500 mL	1474949
Water, deionized	varies	4 L	27256

Required apparatus

Description	Unit	Catalog number
Beaker, 600-mL, polypropylene	each	108052
Bottle, wash, 500-mL	each	62011
Cylinder, graduated, 500 mL, polypropylene	each	108149
Pipet, TenSette, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet	50/pkg	2185696
*sens**ion*** 4 Laboratory pH/ISE Meter	each	5177500
Sodium Combination Electrode, Platinum series, BNC	each	5192500
Stir Bar, $^7/_8$ x $^3/_{16}$ in. (22.2 x 4.8 cm)	each	4531500
Stir Bar, $1^1/_8$ x $^3/_{10}$ in. (28.6 x 7.9 cm)	each	2095355
Select one based on available voltage:		
Stirrer, electromagnetic, 115 VAC, with stand and stir bar	each	4530001
Stirrer, electromagnetic, 230 VAC, with stand and stir bar	each	4530002

Optional reagents

Description	Unit	Catalog number
Sodium Ionic Strength Adjustor (ISA), powder	454 g	4451501

Optional apparatus

Description	Unit	Catalog number
Flask, volumetric, polypropylene, 50 mL	each	1406041
Flask, volumetric, polypropylene, 100 mL	each	1406042
Water Bath, circulating	each	2616300

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Edition 7

Oxygen, Dissolved

DOC316.53.01241

Direct Measurement Method **Method 8157**

0 to 20 mg/L or 0 to 200% saturation **Clark-type Amperometric Sensor**

Scope and Application: For water, wastewater and process water applications

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 417 Instrument-specific information

Meter	Electrode
*sens**ion*™ 5 meters	5197000, 5197003 or 5197015
*sens**ion*™ 8 meters	5197000, 5197003 or 5197015

Before starting the test:

Assemble the probe. Refer to the *Probe assembly* section in this procedure.
Refer to the *sens**ion*** meter manual for instructions on polarizing and zeroing the meter.
Dissolved oxygen probes are continuously polarized when they are connected to the meter. A steady reading will not be seen for 30 to 50 minutes when the probe electrolyte is new or when the probe has been unplugged for more than one hour. Interrupted connections of less than one hour will require 5 to 25 minutes before a stable reading is observed.
While not in use, the probe should be stored with the Calibration and Storage Chamber attached to the end of the probe. The sponge inside the Chamber should be kept moist.
Keep the DO probe at a uniform temperature. When holding the probe, do not touch the metallic button on the side of the probe. The button is a temperature sensor. An inaccurate calibration will result if the temperature of the thermistor is different from the probe membrane.
The displayed % saturation is based on a meter calculation for the equilibrium dissolved oxygen concentration. The calculation uses the sample temperature, salinity, barometric pressure, altitude and measured concentration in mg/L values. Changing the entries in setups 4, 5 or 6 will change the displayed mg/L or % saturation. Refer to the *sens**ion*** meter manual for more information.

Collect the following items:

Description	Quantity
Dissolved oxygen probe	1
*sens**ion*** 6 or 8 meter	1
Filling solution, dissolved oxygen	2 mL
Calibration and storage chamber	1

Collect the following items: (continued)

Description	Quantity
Membrane for dissolved oxygen probe	1
Beaker, polypropylene, 100-, 250-, 400- or 600-mL	1

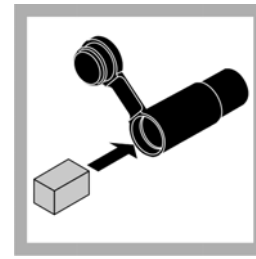
See *Consumables and replacement items* for reorder information.

Dissolved oxygen, amperometric sensor method

1. At least one hour before measurement, polarize the probe by connecting it to the meter.

2. Zero the Dissolved Oxygen meter before calibration when measuring dissolved oxygen levels less than 1 mg/L or 10% saturation. Refer to *Zeroing the probe*.

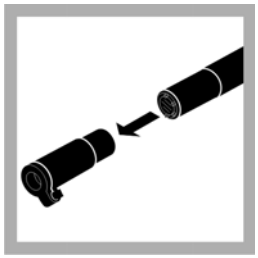
3. Secure the probe cable to the calibration and storage chamber by wrapping cable through the bottom of the chamber lid before filling with water.



4. Prepare the calibration and storage chamber by holding it under water and squeezing it a couple of times to pull water into the lower chamber through the inlet. Alternately, open the bottom of the chamber and insert a damp sponge.

New sponges will be compressed. Add water to expand them. Avoid completely filling the lower chamber with water.

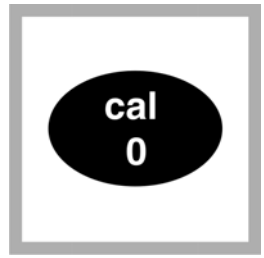
Dissolved oxygen, amperometric sensor method (continued)



5. Insert the DO probe into the calibration and storage chamber. The probe tip must not be flooded with water or be holding a drop of water on the membrane.

6. Allow at least ten minutes for the atmosphere in the chamber to reach a steady state.

To speed up probe stabilization, squeeze the lower chamber a couple of times to force water saturated air into the chamber.



7. Press **CAL**. Edit the barometric pressure. Press **ENTER** and enter the current altitude and press **ENTER**.

Refer to the *Adjusting barometric pressure and altitude* table.

8. The display will show the current value for sample salinity (‰).

Set the salinity to zero (0‰).

9. Press **ENTER**. The display will show 100%.

Press **READ**. When the calibration is complete, the meter will return to measurement mode.

10. Add the weight assembly to the probe if required (3- or 15-m cable versions only).

11. If sample salinity has been measured using an Electrolytic Conductivity Meter, enter the value in parts per thousand.

12. Insert the probe into the sample. The probe must be deep enough to cover the thermistor (metallic button) located on the side of the probe.

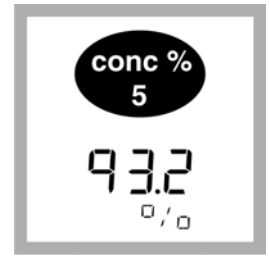
Dissolved oxygen, amperometric sensor method (continued)

13. Agitate the probe in the sample to dislodge air bubbles from the sensing area of the probe tip.

14. Stir the sample vigorously with the probe or use a stir stand and stir bar (for laboratory use).

When measuring deep bodies of water, create sufficient flow across the probe tip by pulling on the cable to move the probe up and down (for field use).

15. When the reading on the meter stabilizes, record or store the value in the meter memory.

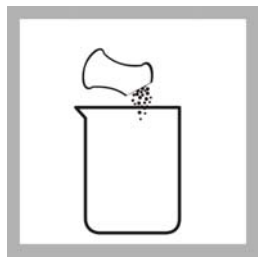


16. Change the display from concentration in mg/L to % saturation if % saturation is required.

For high Sodium concentration samples

1. Pour the 400 mL sample into a 600-mL beaker. Add a magnetic stir bar and put the beaker on an electromagnetic stirrer. Stir at a moderate rate.

If the electrode is in a sample of high sodium concentration, errors may result and reconditioning to lower sodium levels will be required.



2. Add the contents of one Sodium Ionic Strength Adjustor Powder Pillow.

3. Put the electrode into the sample.

Wait 10 minutes to allow electrode to condition to the low level sodium in solution.

4. When the measurement stabilizes, record the sample concentration value.

Barometric pressure and altitude adjustment

Table 418 Adjusting barometric pressure and altitude

Enter a new barometric pressure when the barometric pressure or the altitude of the instrument changes using one of the methods below:	
Sea level equivalent	**True barometric pressure**
1. Obtain the sea level equivalent barometric pressure from TV, radio or a local airport.	**1.** Obtain the true barometric pressure from a nearby mercury barometer.
2. Enter this value into the meter. Refer to the Changing the Barometric Pressure section of the meter manual.	**2.** Enter this value into the meter. Refer to the Changing the Barometric Pressure section of the meter manual.
3. Enter the local altitude. Refer to the Adjusting the Altitude section of the meter manual.	**3.** Enter the altitude as **0** feet or meters. Refer to the Adjusting the Altitude section of the meter manual.

Probe assembly

1. Hold the membrane module cap in a vertical position. Fill the module cap about 2/3 of the way full with Dissolved Oxygen Electrolyte Filling Solution.
2. Hold the DO probe in a vertical position with the tip down. Gently screw the module cap onto the tip. Electrolyte should leak out of the threads.

Note: *If electrolyte does not leak out of the threads, air may remain inside the module cap. Repeat this procedure using more filling solution.*

3. Attach the DO probe cable connector to the input connector at the top of the meter. Refer to the *sens**ion*** 6 Dissolved Oxygen Meter Instruction Manual for additional information.

Probe polarization

Dissolved oxygen probes are continuously polarized when they are connected to the *sens**ion*** meter. A steady reading will not be seen for 30 to 50 minutes when the probe electrolyte is new or when the probe has been unplugged for more than one hour. Interrupted connections of less than one hour will require 5 to 25 minutes before a stable reading is observed.

While not in use, the probe should be stored with the calibration and storage chamber attached to the end of the probe. Keep the sponge inside the chamber moist.

Zeroing the probe

Zeroing the *sens**ion*** 6 Dissolved Oxygen meter is necessary only when measuring dissolved oxygen levels less than 1 mg/L or 10% saturation. A new DO probe can generate a 0.02 to 0.05 mg/L positive error in an oxygen-free (anoxic) solution. If this level of error cannot be tolerated, zero the meter using the following procedure. Zero the meter also after replacing the sensing membrane or changing the internal filling solution.

Procedure

1. Measure about 150 mL of sample or deionized water into a 250-mL beaker. Place a magnetic stir bar in the beaker.
2. Add 0.25 g sodium sulfite or the contents of one Silica 3 Reagent Powder Pillow to the water. Stir to dissolve the reagent.
3. Catalyze the reduction of dissolved oxygen by adding 0.1 mL of a 1000 mg/L Cobalt Standard solution to the water.

4. Place the probe in the stirring sample for at least 10 minutes. This solution is effective for 30 minutes or more.
5. Press the CAL key. The **CAL** icon will appear in the upper left corner of the display.
6. Press the **READ ENTER** key three times to skip to the display showing **100%**.
7. Press the **0** key on the keypad then press **READ ENTER**.
8. The meter shows **Stabilizing...** while the readings are taken. When the meter's zero DO criteria have been met, it will return to the read mode. The meter will not exit the zeroing routine until the meter's zero criteria have been met.
9. When the meter cannot complete the zeroing procedure, it will begin to beep and show the faulty probe icon. If the meter does not complete the zeroing procedure and exits to the reading mode, add additional sodium sulfite and cobalt standard solution to the stirring water. Otherwise, press **EXIT** to return to one display screen at a time and leave the calibration routine without completing the zeroing procedure.

Interferences

Oxidizing gases such as chlorine, chlorine dioxide, sulphur trioxide and bromine can react at the cathode to produce positive interferences. Reducing gases such as hydrogen, hydrogen sulfide, sulfur dioxide and boranes can react at the anode. After exposure to reducing gases, the user may need to clean the anode and replace the internal filling solution and membrane cap.

Sample collection, preservation and storage

- Analyze samples in-situ, if possible.
- Collect samples in 300 mL glass BOD bottles. Fill the bottles completely.
- Analyze samples immediately. Do not store samples.

Accuracy check

1. Return the electrode to the calibration and storage chamber. The chamber should contain a wet sponge or a small amount of water.
2. Allow at least 10 minutes for stabilization.
3. Enter the current barometric pressure and altitude into the meter. Refer to the meter manual.
4. The meter should display 100% saturation. If not, recalibrate the meter.

Method performance

Refer to the electrode and meter manual to determine the method performance.

Summary of method

The Dissolved Oxygen Probe is a Clark-type amperometric sensor used to measure dissolved oxygen in aqueous solutions. It consists of an anode/cathode electrode system and potassium chloride-based electrolyte, separated from the sample by a replaceable oxygen-permeable Teflon® membrane. At a constant temperature, the electric current varies linearly with the oxygen concentration of the solution. A built-in thermistor provides automatic temperature compensation when using the *sens**ion*** Dissolved Oxygen Meters. The unit % Dissolved Oxygen is dependant on the temperature and salinity of the sample and the barometric pressure of the environment where the measurement is taken.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Filling Solution, Dissolved Oxygen	59 mL	2759126

Required apparatus

Description	Unit	Catalog number
*sens**ion*** 6 meter, 115 VAC	each	5185000
*sens**ion*** 8 meter, 115 VAC	each	5455000
Cable, Dissolved Oxygen Probe, 1 meter	.each	.5197000
Cable, Dissolved Oxygen Probe, 3 meter	each	5197003
Cable, Dissolved Oxygen Probe, 15 meter	each	5197015
Barometer, Digital	.each	.2758400
Batteries, AA	4/pkg	1938004
BOD Accessory Kit includes funnel and spacer for Dissolved Oxygen Probe	each	5197100
Calibration Storage Chamber, Dissolved Oxygen Probe	each	5197400
Dissolved Oxygen Service Kit, includes 2 membranes, fill solution, polishing cloth, 2 sponges	each	5196800

Required apparatus (continued)

Description	Unit	Catalog number
Docking Station, external, 115 V, N. American style plug	each	5187501
Docking Station, external, 230 V, European style plug	each	5187502
Membranes, for Dissolved Oxygen Probe	2/pkg	5197300
Weight Assembly	each	5196900

Optional reagents and apparatus

Description	Unit	Catalog number
Cobalt Standard Solution, 100 mg/L	100 mL	2150342
Silica 3 Reagent Powder Pillows (contains sodium sulfite)	100/pkg	27169
Sodium Sulfite	454 g	19501

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Edition 7

Oxygen Demand, Biochemical

DOC316.53.01242

Dilution Method[1]

Method 10230

Scope and Application: For water and wastewater.

LBOD Measurement

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater* and from Klein, R.L.; Gibbs, C. *Journal of Water Pollution Control Federation*, 1979, 51(9), 2257. USEPA recommended for compliance monitoring. Meets ASTM 888-05 (C)

Test preparation

Before starting the test:

The BOD test is a 5-day test. Follow all steps carefully to make sure that the test does not have to be repeated. The IntelliCAL™ LBOD probe measures dissolved oxygen in a 300 mL BOD bottle.
The dilution water for this test must not have an oxygen demand or any toxins. When incubated for 5 days at 20 °C, the dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L.
Carbonaceous BOD (CBOD) can be determined by the addition of nitrification inhibitor. A test for CBOD is recommended for biologically treated effluents, samples seeded with biologically treated effluents and river water.

Collect the following items:

Description	Quantity
BOD bottles, 300-mL, glass, with glass stoppers and plastic caps	6
Dilution water containing nutrient buffer and seed (see *Dilution water preparation*)	varies
HQ40d or HQ30d meter with IntelliCAL LBOD101 probe	1
Nitrification inhibitor (for CBOD only)	1 bottle
Pipet, seriological	1
Incubator	1

See *Consumables and replacement items* for reorder information.

Dilution method

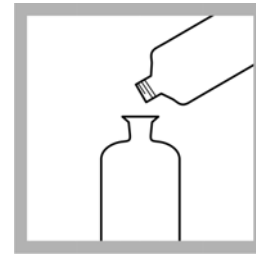
1. Prepare the dilution water using a BOD Nutrient Buffer Pillow. See *Dilution water preparation*.

2. Select the sample volumes. See *Sample size selection*.

3. Stir the sample gently with the pipet. Use the pipet to add the minimum sample volume to the first BOD bottle.

Add the remaining four sample volumes to four more BOD bottles.

When analyzing disinfected samples or industrial effluents, refer to *Interferences*.

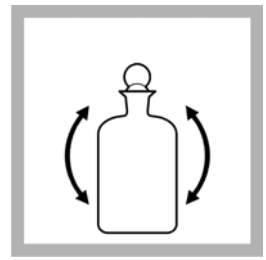


4. Fill an additional BOD bottle with dilution water only. This will be the dilution water blank.

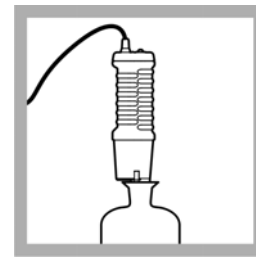
5. If the test is for CBOD, add two portions of Nitrification Inhibitor (approximately 0.16 g) to each bottle.

The oxidation of nitrogen compounds will be prevented. Report results as CBOD.

6. Fill each bottle to just below the lip with dilution water. Allow the dilution water to flow down the sides of the bottle to prevent air bubbles from becoming trapped in the bottle.



7. Stopper the bottles carefully to prevent air bubbles from becoming trapped. Press down on the stopper and invert the bottles several times to mix.



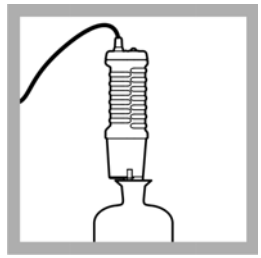
8. Probe calibration is required before initial and final BOD readings. Refer to the *Calibration* section of this procedure.

Be sure to measure the DO of the blank.

An initial DO measurement is not necessary when the graphical method (not for reporting) is used for calculation.

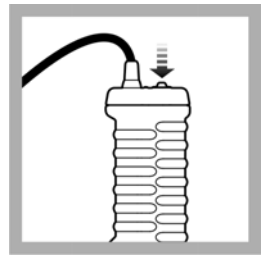
Dilution method (continued)

9. Rinse the LBOD probe with deionized water.

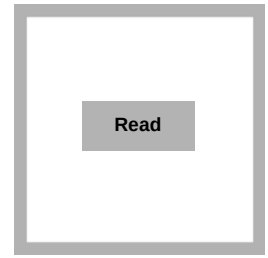


10. Place the LBOD Probe in the BOD Bottle containing the sample.

Make sure there are no air bubbles trapped under the probe.



11. Engage the stir paddle on the LBOD probe by pushing the button on the top of the probe. The green indicator light on the top of the probe will illuminate when the stirrer is running.



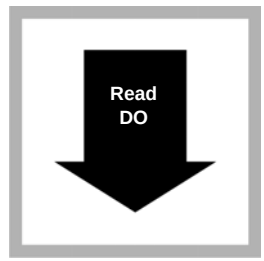
12. Press the key under **READ**. After the measurement has stabilized, the dissolved oxygen value will show on the display.

13. Record the value.

Data is stored automatically in the Data Log when Press to Read or Interval is selected in the Setup Measurement Mode. When Continuous is selected, data will only be stored when the **GREEN/RIGHT** key under Store is pressed.

14. Remove the LBOD Probe from the bottle and stopper the bottles carefully to prevent air bubbles from becoming trapped. Add dilution water to the lip of each BOD Bottle to make a water seal.

Repeat Steps 9–14 for each BOD bottle.



15. After five days, measure the remaining dissolved oxygen concentration in each bottle.

At least 1.0 mg/L DO should be left in each BOD bottle.

16. Calculate the BOD value (see *BOD calculation—standard methods* or *BOD calculation—graphical method*).

Calibration

Water-saturated air calibration

1. Fill a BOD bottle ¾ full with water (225 mL). If a 0% calibration point is required, refer to the *Sulfite correction* section.
2. Put the BOD stopper in the BOD bottle and shake vigorously for about one minute to saturate the air with water.

3. Remove the stopper. Put the Intellical LBOD Probe into the BOD bottle for several minutes to reach equilibrium. Inspect the LBOD probe sensor cap surface to make sure it is dry. If the sensor cap is wet, carefully dry the cap with a non-abrasive cloth.
4. Make sure the meter is in the measurement screen. Press **CALIBRATION**.

   Note: *For the HQ40d meter with two probes connected, make sure the meter is in the single scree LDO101 mode.*

5. Press **READ**. When the measurement has stabilized, the calibrated measurement will show on the screen. The standard value will be highlighted.
6. Press **DONE** to view the calibration summary. The slope value is the comparison between the latest calibration and the factory calibration expressed as a percentage.

   Note: *If the calibration slope does not meet the acceptance criteria, the display will show "Slope out of range". Let the probe stand in water-saturated air for several minutes. When the probe reaches equilibrium, press* ***READ****.*

7. Press **STORE** to accept the calibration and return to the measurement mode. The calibration record is stored in the data log.

Note: *A successful calibration will show "OK" in the measurement screen.*

Sulfite correction

1. Fill a BOD bottle full with deionized water.
2. Add 300 mg of sodium sulfite to the bottle.
3. Add a small crystal of cobalt chloride.
4. Put the stopper in the BOD bottle and invert several times to mix the chemicals.
5. Put the LBOD probe in the bottle and engage the stirrer. This will help speed up the calibration. When the meter reaches a stable reading, press the calibration button on the meter.
6. After the 0% saturated message is displayed press **STORE**. After using sulfite, be sure to clean the probe thoroughly.
7. To clean the sulfite off of the probe, put the LBOD probe in a BOD bottle full of water, activate the stirrer and run for 10 minutes to remove sulfite residue.

Dilution water preparation

The dilution water must be prepared very carefully to make sure that no source of oxygen demand or toxins are added. The water that is used to prepare the dilution water must be of very high quality. The water must not have any organic compounds or any toxic compounds such as chlorine, copper and mercury.

Use the following guidelines to make sure the dilution water is of high quality.

Guidelines

- Use distilled water from an alkaline permanganate distillation for the best results.
- Do not use deionized water from ion exchange columns. The resins in the cartridges (especially new cartridges) will occasionally release organic materials that have an oxygen demand. In addition, bacteria can grow on the columns and contaminate the dilution water.
- Store the distilled water in clean jugs in an incubator at 20 °C. Shake the jugs to saturate the water with air or cap the jugs loosely and store for 24 hours or more.

- A small aquarium pump or air compressor can be used to saturate the water with air. Make sure that the air is filtered and that the filter does not grow bacteria.
- Add the nutrients and seed (if necessary) to the distilled water immediately before the test.
- The dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L when incubated for 5 days at 20 °C.

Procedure

1. Prepare and store the distilled water at 20 °C (see *Guidelines*).
2. Select a BOD nutrient buffer pillow from the *BOD nutrient buffer pillows* table.
3. Shake the pillow to mix the contents.
4. Add the contents of the pillow to the distilled water. Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.
5. If the sample is known to be low in bacteria, for example industrial waste or sewage that has been disinfected, add 3 mL of bacterial seed to each liter of the dilution water. Use raw sewage for the bacterial seed. Allow the sewage to stand undisturbed at 20 °C for 24 to 36 hours before use. Pipet from the upper portion of the sewage. Make sure to measure the BOD of the seed so that it can be subtracted from the BOD of the sample.

Table 419 BOD nutrient buffer pillows

Volume of dilution water to prepare	BOD nutrient buffer pillow catalog no.
300 mL (add pillow to each BOD bottle)	1416066
3 liters	1486166
4 liters	2436466
6 liters	1486266
19 liters	1486398

Note: *To prepare dilution water by the conventional method, pipet 1 mL of each of the following solutions per liter of distilled water at 20 °C: Calcium Chloride Solution, Ferric Chloride Solution, Magnesium Sulfate Solution and Phosphate Buffer Solution. Cap the bottle and shake vigorously for one minute. The Phosphate Buffer Solution should be refrigerated to decrease the rate of biological growth. Use care with all solutions to avoid contamination.*

Sample size selection

Make an estimation of the sample volumes that are necessary for the test. At least 2.0 mg/L of dissolved oxygen (DO) should be consumed during the test and at least 1.0 mg/L DO should be left in the BOD bottle.

Samples such as raw sewage will have a high BOD. Small sample volumes must be used because large samples will consume all of the oxygen. Samples with a low BOD must use larger sample volumes to make sure that enough oxygen is consumed to give accurate results.

The elevation of the laboratory changes the amount of oxygen that can dissolve in water (refer to the *Oxygen values at various altitudes (20 °C)* table). At higher elevations, the amount of oxygen that can dissolve in water decreases, so less oxygen is available to microorganisms.

Procedure

1. Refer to the *Minimum sample volume* table to select the minimum sample volume. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the minimum sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the minimum sample volume is 15 mL.

2. Refer to the *Maximum sample volume* table to select the maximum sample volume. At 1000 feet, with an estimated BOD of 300 mg/L, the largest sample volume is 8 mL. For a BOD of 40 mg/L the maximum volume is 60 mL (also at 1000 feet).
3. Select three other sample volumes between the minimum and maximum volumes so that there are five sample volumes total.

Table 420 Minimum sample volume

Sample type	Estimated BOD (mg/L)	Minimum sample volume (mL)
Strong trade waste	600	1
Raw and settled sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted river waters	6	100
	4	200
	2	300

Table 421 Maximum sample volume

BOD at sea level	BOD at 1000 ft	BOD at 5000 ft	Maximum sample volume (mL)
2460	2380	2032	1
1230	1189	1016	2
820	793	677	3
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

Table 422 Oxygen values at various altitudes (20 °C)

Altitude (ft)	Oxygen value (mg/L) in water saturated with air
Sea level	9.2
1000	8.9
2000	8.6
3000	8.2
4000	7.9
5000	7.6
6000	7.4

BOD calculation—standard methods

Use the Standard Methods calculation when the results must be reported to a regulatory agency.

When dilution water is not seeded:

$$BOD_5,\ mg/L = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$BOD_5,\ mg/L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where:

BOD_5 = BOD value from the 5-day test

D1 = DO of diluted sample immediately after preparation, in mg/L

D2 = DO of diluted sample after 5 day incubation at 20 °C, in mg/L

P = Decimal volumetric fraction of sample used

B1 = DO of seed control before incubation, in mg/L

B2 = DO of seed control after incubation, in mg/L

f = ratio of seed in diluted sample to seed in seed control =

(% seed in diluted sample)/(% seed in seed control) OR

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification inhibitor was added.

Averaged results are acceptable if more than one sample dilution meets all of the following criteria:

- The remaining DO is at least 1 mg/L
- The final DO value is at least 2 mg/L lower than the initial DO value
- There is no evidence of toxicity at higher sample concentrations
- There are no obvious anomalies

BOD calculation—graphical method

Important Note: *The Graphical Method cannot be used when the results must be reported to a regulatory agency.*

1. Plot the mg/L dissolved oxygen (DO) remaining in each diluted sample versus the mL sample taken. Draw the best straight line through the plotted points. Refer to *Dissolved Oxygen per mL of Sample*.

Note: *An erroneous point is visually evident at this time and can be disregarded. However, at least three points should be on the line or very close to it. For unseeded dilution water, the line should cross the mg/L oxygen remaining scale near or below the oxygen saturation value for the altitude of the laboratory as discussed in Dilution water preparation.*

2. To calculate the BOD, use the following equation which is mathematically equivalent to the BOD equation in Standard Methods.

mg/L BOD = (A x 300) – B + C

where:

A = the slope

The slope of the line is equal to the mg/L DO consumed per mL of sample taken. Take any point on the line and subtract the mg/L DO remaining at that point from the mg/L DO where the line crosses the DO scale (Y intercept, mg/L DO remaining). Divide the difference by the mL of sample at the point chosen.

300 = the volume of the BOD bottle

B = the Y intercept

This is the DO value where the line crosses the “DO remaining” scale. (This should be very close to the actual dilution water blank value.)

C = the sample DO

This is the DO of the undiluted sample.

Another way to write this equation is:

mg/L BOD = (Slope x 300) – Y intercept + Sample DO

Note: *If the best straight line is obtained by linear regression through use of a calculator, the sign (-) of the slope must be changed (+) before multiplying by 300.*

Example:
The mg/L DO remaining was determined for a series of four dilutions of domestic sewage after five days of incubation. Results were as follows:

mL of sample taken	mg/L DO remaining
2.0	7.50
3.0	6.75
6.0	4.50
9.0	2.25

The DO values were plotted versus the mL of sample taken and a straight line drawn as in *Dissolved Oxygen per mL of Sample*. If a set of BOD dilutions is run correctly with a homogeneous sample, a graph of the mg/L DO remaining versus the sample volume would result in a straight line. The value where the line intersects the y-axis is equal to the DO content of the dilution water after incubation, although this is not actually measured. In this case, it was equal to 9.0 mg/L and the DO of the domestic sewage sample was assumed to be zero. If another type of sample is used, the DO of an undiluted sample should be measured either by the Winkler titration or potentiometrically.

The American Public Health Association formula for calculating BOD also can be written as follows (not approved for reporting purposes):

$$\frac{\text{mg/L DO remaining w/smaller sample volume} - \text{mg/L DO remaining w/larger sample volume}}{\text{mL of larger sample volume} - \text{mL of smaller sample volume}} \times 300 - DO_D + S = \text{mg/L BOD}$$

Using this information in the example:

mg/L DO remaining with smaller sample volume = 7.50

mg/L DO remaining with larger sample volume = 2.25

mL of larger sample volume = 9.0

mL of smaller sample volume = 2.0

300 = volume (mL) of BOD bottle

DO_D = mg/L DO of dilution water = 9.0

S = mg/L DO of sample = assumed in this case to be zero

Therefore:

$$\frac{7.50 - 2.25}{9.0 - 2.0} \times 300 - 9 + 0 = \text{mg/L BOD} = 216 \text{ mg/L BOD}$$

Using the equation below:

(slope x 300) – Y-Intercept + sample DO = mg/L BOD

To determine slope, arbitrarily select point A in Figure 1. At this point the mg/L DO remaining is equal to 3.0 mg/L. The mL of sample at this point is 8 mL.The difference between the y-intercept of 9.0 mg/L and 3.0 mg/L equals 6 mg/L; 6 mg/L divided by 8 mL = 0.75 mg/L per mL.

slope = 0.75 mg/L per mL

Y intercept = 9.0 mg/L

sample DO = 0 (Because the sample is domestic sewage, this is assumed to be zero.)

Therefore:

(0.75 x 300) – 9.0 + 0 = mg/L BOD = 216 mg/L BOD

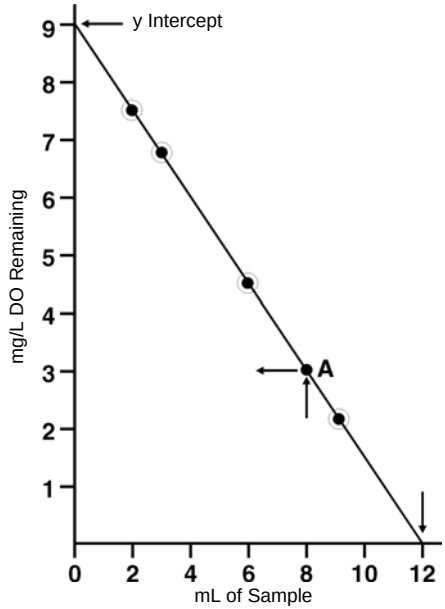


Figure 35 Dissolved Oxygen per mL of Sample

Interferences

Many chlorinated and industrial effluents require special handling to ensure reliable BOD results. Usually, careful experimentation with the particular sample will indicate what modifications should be made to the test procedure.

Toxins in the sample will adversely affect any microorganisms present and result in lower BODs.

To eliminate small amounts of residual chlorine, allow the sample to stand for one to two hours at room temperature. For larger quantities, determine the amount of sodium thiosulfate to add to the sample as follows:

c. Measure 100 mL of sample into a 250-mL Erlenmeyer flask. Using a 10-mL serological pipet and a pipet filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100-g/L, to the flask.

d. Add three full droppers of Starch Indicator Solution and swirl to mix.

e. Fill a 25-mL buret with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.

f. Calculate the amount of 0.025 N Sodium Thiosulfate Standard Solution to add to the sample:

$$\text{mL 0.025 N sodium thiosulfate required} = \frac{\text{mL titrant used x volume of remaining sample}}{100}$$

g. Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample. Mix thoroughly. Wait 10 to 20 minutes before running the BOD test.

To eliminate the effect of phenols, heavy metals or cyanide, dilute the sample with high quality distilled water. Alternately, the seed used in the dilution water may be acclimatized to tolerate such materials. Acclimatize seed as follows:

a. Fill a one-gallon stainless steel or plastic container with domestic sewage and aerate for 24 hours. Allow the heavier material to settle.

b. After settling for one hour, siphon off three quarts of material and discard.

c. Fill the container with a mixture of 90% sewage and 10% wastes containing the toxic material.

d. Aerate for 24 hours. Repeat steps b and c with increasing amounts of waste until the container holds 100% toxic waste material.

Optimum pH for the BOD test is between 6.5 and 7.5. Adjust samples to pH 7.2 with Phosphate Buffer Solution or 1 N Sulfuric Acid or Sodium Hydroxide Standard Solution if the pH is not in this range.

Cold samples may be supersaturated with oxygen and will have low BOD results. Fill a one-quart bottle about halfway with cold sample and shake vigorously for two minutes. Allow sample temperature to reach 20 °C before testing.

Accuracy check

Standard solution method
Required for accuracy check:

- BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL (300-mg/L of glucose and 300-mg/L of glutamic acid)
- Seeded dilution water
- 4 BOD bottles
- 1.0–4.0 mL Class A volumetric pipets
- TenSette Pipet

1. Open the standard solution ampule.
2. Use a pipet to add 1.00, 2.00, 3.00 and 4.00 mL of standard into four BOD bottles.
3. Fill the bottles with seeded dilution water and measure the DO concentration.
4. Incubate the bottles at 20 °C for five days.
5. Measure the DO remaining in each bottle.
6. Calculate the BOD value (refer to *BOD calculation—standard methods* or *BOD calculation—graphical method*).
7. Divide the value by two. The result for comparison with Standard Methods should be 198 (± 30.5) mg/L.

   Note: *The result must be divided by 2 to correspond with values reported in Standard Methods because the Standard Methods procedure uses 150 mg/L each of glucose and glutamic acid.*

Method performance

The following statements are true for dissolved oxygen when the measurement is below 10 mg/L DO and the temperature is kept between 10 and 30 °C for a single probe.

Instrument	Standard	Precision 95% Confidence Limits of Distribution
LBOD101	7.94–8.06 mg/L DO	7.97–8.03 mg/L DO

Summary of method

Biochemical Oxygen Demand (BOD) is a measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters. The BOD test is of limited value in measuring the actual oxygen demand because temperature change, biological population, water movement, sunlight, oxygen concentration and other environmental factors cannot be reproduced accurately in the laboratory. The BOD test is of greatest value after patterns of oxygen uptake for a specific effluent and receiving water have been established.

The BOD test is performed by incubating a sealed wastewater sample (or a prepared dilution) for the standard five-day period and then determining the change in dissolved oxygen content. The BOD value is then calculated from the results of the dissolved oxygen tests.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
BOD Nutrient Buffer Pillows, for 3 liters of dilution water	1 pillow	50/pkg	1486166

Required apparatus

Description	Quantity/Test	Unit	Catalog number
BOD Bottle, glass-stoppered, 300-mL	6	each	62100
BOD Bottle Cap	6	6/pkg	241906
Bottle, wash, 500-mL	1	each	62011
Clippers, large	1	each	96800
HQ40d meter	1	each	HQ40d
OR			
HQ30d meter	1	each	HQ30d
IntelliCAL LBOD probe	1	each	LBOD10101
Pipet, seriological:			
Pipet, serological, 1-mL	1	each	919002
Pipet, serological, 5-mL	1	each	53237
Pipet, serological, 10-mL	1	each	53238
Pipet Filler	1	each	1218900

Recommended standards

Description	Unit	Catalog number
BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL	16/pkg	1486510

Optional reagents and apparatus

Description	Unit	Catalog number
BOD Nutrient Buffer Pillows		
for 300 mL of dilution water	50/pkg	1486166
for 4 liters of dilution water	50/pkg	2436466
for 6 liters of dilution water	50/pkg	1486266
for 19 liters of dilution water	25/pkg	1486398
Buffer Solution, APHA, for BOD, pH 7.2, phosphate type	1 L	43153
Calcium Chloride Solution, APHA, for BOD	1 L	42853
Ferric Chloride Solution, APHA, for BOD	1 L	42953
Magnesium Sulfate Solution, APHA, for BOD	1 L	43053
Nitrification Inhibitor	35 g	253335
Dispenser Cap, for Nitrification Inhibitor	each	45901
Potassium Iodide Solution, 100-g/L	500 mL	1228949
Sodium Hydroxide, pellets, ACS	500 g	18734
Sodium Hydroxide Standard Solution, 1.000 N	100 mL MDB	104532
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	35253
Starch Indicator Solution	100 mL MDB	34932
Sulfuric Acid Standard Solution, 0.020 N	1 L	20353
Sulfuric Acid Standard Solution, 1.000 N	1 L	127053
Up-Grade Kit, HQd Accessories, LBOD Probe	each	LBOD10130
Replacement LBOD Sensor cap, with I-button	each	5838000
Replacement Stirrer assembly	5/pkg	5850800
Field Kit, includes: protective glove, 2 standard probe holders and 5 120-mL sample cups	each	5825800
Keyboard (HQ40d meter only)	each	LZV582
Citizen PD-24 USB Handy printer, 115 VAC	each	5835800
Color Coded Probe Clips (5 color coded sets), 5 sets	10/pkg	5818400

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Edition 7

Oxygen, Dissolved

DOC316.53.01243

Direct Measurement Method **Method 10360**

(0.1 to 20.0 mg/L or 1 to 200% saturation) **LDO Probe**

Scope and Application: For water, wastewater and process water applications

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 423 Instrument-specific information

Meter	Probe
HQd meters	LDO101

Before starting the test:

Before attaching probes to the HQd meter for the first time, set the meter time and date.
Refer to the probe instructions for probe preparation.
For probes that are continuously immersed in aqueous solutions, condition the sensor cap for 72 hours.
When an IntelliCAL™ probe is connected to a HQ30d or HQ40d meter, the meter automatically recognizes the measurement parameter and is ready for use.
The IntelliCAL LDO101 probes automatically compensate for barometric pressure, elevation and temperature.
The LDO probe is calibrated at the factory. For more accurate results, manual calibration is recommended. Refer to the *Calibration* section of this procedure.
Salinity affects the concentration of dissolved oxygen in the sample. To correct for salinity effects, refer to Modifying LDO Measurement Options in the meter manual.

Collect the following items:

Description	Quantity
HQd meter	1
IntelliCAL LDO101 probe	1
Sensor cap for HQd with I-button	1
Shroud	1
BOD bottle, 300-mL or Erlenmeyer flask, 250-mL	1
Beaker, polypropylene (100-, 250-, 400- or 600-mL)	1

See *Consumables and replacement items* for reorder information.

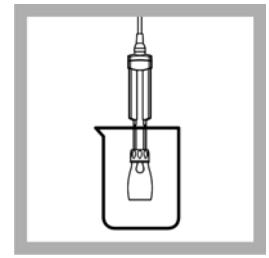
Method Name for powder pillows

1. Prepare the probe. Refer to the probe instructions.

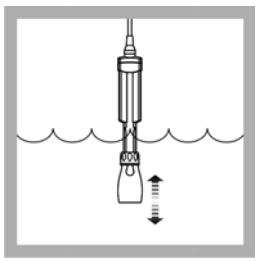
2. Connect the probe to the meter.

3. Calibrate the probe. Refer to the *Calibration* section of this procedure.

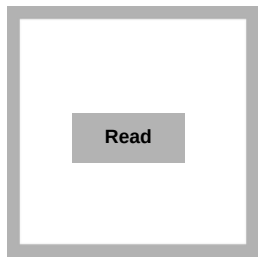
Go to step 4 for laboratory tests. Go to step 5 for field tests.



4. Laboratory tests: Immerse the probe in the beaker containing the sample solution. Move the probe up and down and tap it on the beaker to remove bubbles from the probe.



5. Field tests: Immerse the probe directly into the sample. Move the probe up and down to remove bubbles from the probe.



6. Press **READ** to store data in the data log.

Calibration

The LDO probe is calibrated at the factory. For more accurate results, manual calibration is recommended.

1. Remove the shroud from the probe body.
2. Add a small amount of water (about 1 cm) to the bottom of narrow-neck bottle, such as a BOD bottle.

   Note: *Use a wider neck bottle or flask (for example, a 250-mL Erlenmeyer flask) for the rugged probe.*
3. Insert a stopper and shake vigorously for several minutes.
4. Remove the stopper. If the sensor cap surface is wet, carefully dry the cap with a nonabrasive cloth, then put the probe in the bottle. Allow several minutes for the probe to reach equilibrium.

5. Make sure the meter is in the measurement screen. Press the **CALIBRATION** key.

   Note: *For HQ40d meters with two probes attached, the display must be in the single screen LDO101 mode.*

6. Press **READ**. When the measurement is stable, the calibrated measurement will show on the display. The standard value will be highlighted on the display.

7. Press **DONE** to view the calibration summary. The slope value is the comparison between the latest calibration and the factory calibration expressed as a percentage.

   Note: *If the calibration slope does not meet the acceptance criteria, the display will show "Slope out of range". Let the probe stand in water-saturated air for several minutes. When the probe reaches equilibrium, press* ***READ****.*

8. Press **STORE** to accept the calibration and return to the measurement mode. The calibration record is stored in the data log.

Note: *A successful calibration will show "OK" in the measurement screen.*

Interferences

There are no significant interferences with the LDO technology.

The IntelliCAL LDO101 probes are designed for water and wastewater applications, but can be used for other applications. Some organic solvents may damage the sensor cap and probe body.

Sample collection, preservation and storage

- Analyze samples in-situ, if possible.
- Collect samples in an appropriate container. Fill completely and analyze immediately.
- Do not store samples.

Accuracy check

1. Return the electrode to a water-saturated air environment.
2. Allow at least 10 minutes for stabilization.
3. Read the % saturation on the right side of the measurement mode screen. The meter should display 100% saturation. If not, allow additional time for the air to reach water saturation or calibrate the probe.

Method performance

The following statements are true for dissolved oxygen when the temperature is kept between 10 and 30 degrees C.

Method	Standard	Precision 95% Confidence Limits of Distribution	Accuracy Concentration change per 0.010 Abs change
10360	8.00 mg/L DO	7.95–8.05 mg/L DO	7.90–8.10 mg/L DO
10360	15.00 mg/L DO	14.90–15.10 mg/L DO	14.80–15.20 mg/L DO

Summary of method

The oxygen sensor is made up of a clear, oxygen impermeable hard substrate. An oxygen sensitive luminescent dye, along with a scattering agent, is pad-printed on the substrate. A final overlay of dark pigment is added to prevent stray light from entering the measurement cell. The luminescent dye emits red light when exposed to blue light. The scattering agent distributes the emitted light throughout the sensor matrix and contributes to the opacity of the sensor. Pulses from a red LED serve as an internal reference. The duration of the luminescence is proportional to the concentration of dissolved oxygen in the sample.

Consumables and replacement items

Required apparatus (select one)

Description	Quantity/Test	Unit	Catalog number
HQ40d multi-parameter meter, dual input	1	each	HQ40d53000000
HQ30d multi-parameter meter, single input	1	each	HQ30d53000000

Required probes (select one)

Description	Unit	Catalog number
LDO Probe, standard, with 1 m cable	each	LDO10101
LDO Probe, standard, with 3 m cable	each	LDO10103
LDO Probe, rugged, with 5 m cable	each	LDO10105
LDO Probe, rugged, with 10 m cable	each	LDO10110
LDO Probe, rugged, with 15 m cable	each	LDO10115
LDO Probe, rugged, with 30 m cable	each	LDO10130

Optional apparatus

Description	Unit	Catalog number
AC Power Adapter for HQd meters (included w/ HQ40d)	each	5826300
BOD bottle, 300 mL	each	62100
BOD bottle, 300 mL	6/pkg	62106
Citizen PD-24 USB Handy printer, 115 VAC	each	5835800
Color Coded Probe Clips (5 color coded sets) 5 sets	10/pkg	5818400
Depth Markers for Rugged LDO probe only	10/pkg	5828610
Erlenmeyer flask, 250 mL	each	2089846
Field Kit (Includes glove kit, 2 probe holders and 5 120 mL sample cups)[1]	each	5825800
Glove kit only for HQd meters	each	5828700
Probe Holder for HQd meter, IntelliCAL Standard probes only	each	5829400
Replacement Sensor cap w/ I-button	each	5811200
Replacement Shroud kit Rugged LDO probe	each	5825900
USB Keyboard for HQd meters (must have 5813400 & 5826300)	each	LZV582
USB/DC Adapter for HQd meters (must have 5826300, inc w/HQ40d)	each	5813400

[1] Included with HQ40d

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Edition 7

Oxidation Reduction Potential (ORP)

DOC316.53.01244

Direct Measurement Method **Method 10228**

(–2000 mV to 2000 mV) **ORP Electrode**

Scope and Application: For drinking water, wastewater and process water applications

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 424 Instrument-specific information

Meter	Platinum-series ORP electrode	Gel-filled ORP electrode
*sens**ion***™ 1	5193700	5193900
*sens**ion***™ 2	5193700	5193900
*sens**ion***™ 3	5193700	5193900
*sens**ion***™ 4	5193700	5193900

Collect the following items:

Description	Quantity
*sens**ion*** pH/mV meter	1
Gel-filled or combination ORP electrode	1
Beaker, polypropylene (100-, 250-, 400- or 600-mL)	1
Deionized water	

See *Consumables and replacement items* for reorder information.

Oxidation reduction potential (ORP)

1. Connect the electrode to the meter.

2. Turn on the meter. Press **PHMV** to select mV.

3. Rinse the electrode in deionized water and blot dry.

4. Platinum-series electrodes only: Press the dispenser button on top of the electrode until the electrolyte get is visible at the reference junction.

5. Put the electrode in the sample.

6. When the measurement stabilizes, store or record the mV and temperature readings.

Standard hydrogen electrode (SHE) calculation

For some applications it is customary to report oxidation reduction (redox) potential measurements relative to the standard hydrogen electrode (SHE).

1. Select the value that corresponds to the temperature of the solution measured. Refer to the *Reference electrode potentials* table.
2. Substitute the electrode potential value (C) in the equation and solve for E_{SHE}:

   $E_{SHE} = E_O + C$

   Where:

   E_{SHE} = oxidation reduction potential of the sample relative to the SHE, following the international sign convention.

   E_O = potential developed by the ORP electrode

   C = potential developed by the reference electrode relative to the SHE.

The *Reference electrode potentials* table shows the potentials, C, developed by the reference electrode relative to the standard hydrogen electrode at various temperatures.

Table 425 Reference electrode potentials

Temperature (°C)	Electrode potential in mV (C)
10	221
15	216
20	213
25	208
30	204
35	200
40	196

Interferences

Many factors limit the interpretation of ORP measurements in water. These factors include irreversible reactions, electrode poisoning, the presence of multiple redox couples, very small exchange currents and inert redox couples. ORP measurements in the field correlate poorly with ORP values calculated from the redox couples present. Due to these factors, the interpretation of ORP measurements will be specific to your particular application.

Sample collection, preservation, storage and cleaning

- Analyze samples immediately after collection.
- For best results, minimize contact with the environment and the time between collection and measurement.
- If the electrode is not clean, remove inorganic deposits by immersing the electrode tip in room-temperature 0.1 N HCl for 10 minutes. To remove grease, oil or other organic deposits, immerse the tip in warm water and detergent and swirl gently. After cleaning, rinse with DI water. Repeat *Procedure A* after cleaning.

Accuracy check

Checking the electrode is necessary only when there is evidence of malfunction that cannot be traced to other causes.

Procedure A

1. Open an ampule of Light's Solution or ORP verification solution*. Pour the contents of the ampule into a beaker.
2. Immediately place the ORP Electrode tip into the solution.
3. Verify that the potential is 475 ± 10 mV or the specified ORP value.

Note: *This potential is the standard reduction potential for $Fe^{2+/3+}$ with the reference electrode potential subtracted. The solution is 0.01 M in both Fe^{2+} and Fe^{3+}.*

* See *Optional apparatus*.

Procedure B

1. Prepare solution A (0.1 M potassium ferrocyanide and 0.05 M potassium ferricyanide) as follows:
   a. Weigh out 4.22 g reagent-grade $K_4Fe(CN)_6 \bullet 3H_2O$ and 1.65 g reagent-grade $K_3Fe(CN)_6$. Put the solids in a 100-mL volumetric flask.
   b. Add about 50 mL deionized water and swirl to dissolve the solids.
   c. Dilute to volume with deionized water.
2. Prepare solution B (0.01 M potassium ferrocyanide, 0.05 M potassium ferricyanide and 0.36 M potassium fluoride) as follows:
   a. Weigh out 0.42 g of reagent-grade $K_4Fe(CN)_6? \bullet 3H_2O$, 1.65 g of reagent-grade $K_3Fe(CN)_6$ and 3.39 g of reagent-grade $KF \bullet 2H_2O$. Put the solids in a 100-mL volumetric flask.
   b. Add about 50 mL deionized water and swirl to dissolve the solids.
   c. Dilute to volume with deionized water.
3. Transfer solution A to a 150-mL beaker. Place the electrode in the solution and wait until the reading stabilizes. The potential should be about 234 mV.
4. Rinse the electrode and repeat the measurement with solution B. The potential should be about 66 mV greater in solution B than in solution A.

Summary of method

Redox measurements are made by determining the electron activity of a solution using an inert indicator electrode and a reference electrode. The potential difference between the indicator electrode and the reference electrode equals the redox potential of the system. The Gel-filled ORP and Platinum Series ORP electrodes use a platinum indicator electrode and a silver/silver chloride reference electrode.

Consumables and replacement items

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Select one meter and probe combination:	—	—	—
*sens**ion***™ 1 Portable pH/mV Meter		each	5170010
*sens**ion***™ 2 Portable pH/ISE Meter		each	5172510
*sens**ion***™ 3 Benchtop pH/mV Meter		each	5175010
*sens**ion***™ 4 Benchtop pH/ISE Meter		each	5177510
Gel-filled Combination ORP Electrode, 5-pin	1		5193900
OR			
Platinum Series Combination ORP Electrode, 5-pin	1		5193700

Optional reagents

Description	Unit	Catalog number
Light's Solution, ampules	20/pkg	26125-20
200 mV ORP Solution	500 mL	25M2A1001-115
600 mV ORP Solution	500 mL	25M2A1002-115
Hydrochloric Acid Standard Solution 0.1 N	1 L	1481253
Sodium Hydrochloride Standard Solution, 0.1 N	1 L	19153

Optional apparatus

Description	Unit	Catalog number
Beaker, 100-mL, polypropylene	each	.108042
Beaker, 250-mL, polypropylene	each	108046
Beaker, 400-mL, polypropylene	each	108048
Beaker, 600-mL, polypropylene	each	108052
Beaker, 1000-mL, polypropylene	each	108053
Digital Titrator	each	1690001
Flask, Erlenmeyer, 125-mL	each	50543
Electrode Holder, with electromagnetic stirrer	each	4530001
Electrode Holder	each	4530000
Stir Bar, Magnetic, 22.2 x 7.9 mm	each	2095350

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Edition 7

USEPA Electrode Method — Method 8156

pH Meter

Scope and Application: For drinking water[1], wastewater[2] and process water applications.

[1] Based on Standard Method 4500-H+B, ASTM Method D1293-95 and USEPA Method 150.1

[2] Based on Standard Method 4500-H+B, ASTM Method D1293-84(90)/(A or B) and USEPA Method 150.1

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 426 Instrument-specific information

Meter	Standard probe	Rugged probe[1]
HQ40d	PHC10101, PHC10103 (gel) PHC30101, PHC30103 (liquid)	PHC10105, PHC10110, PHC10115, PHC10130
HQ30d	PHC10101, PHC10103 (gel) PHC30101, PHC30103 (liquid)	PHC10105, PHC10110, PHC10115, PHC10130
HQ11d	PHC10101, PHC10103 (gel) PHC30101, PHC30103 (liquid)	PHC10105, PHC10110, PHC10115, PHC10130
*sens**ion***™ 1	5191000 (platinum) 5193500 (gel) 5194000 (refillable) 5191500 (flat)	—
*sens**ion***™ 3	5191000 (platinum) 5193500 (gel) 5194000 (refillable) 5191500 (flat)	—

[1] Designed for field use.

Before starting the test:

For optimal response time, condition the electrode for several minutes in a solution comparable to the sample in terms of pH and ionic strength.
For rugged IntelliCAL™ electrodes, the shroud may need to be removed before measurement and calibration.
For HQd meters, data is stored automatically when Press to Read or Interval is selected in the Setup Measurement Mode. When Continuous is selected, data will only be stored when the key under **STORE** is pressed. For *sens**ion*** meters, the **STORE** key must be pressed.

Collect the following items:

Description	Quantity
pH meter and probe combination	1
pH buffers (4.0, 7.0, 10.0)	3
Beakers/sample containers	3

See *Consumables and replacement items* for reorder information.

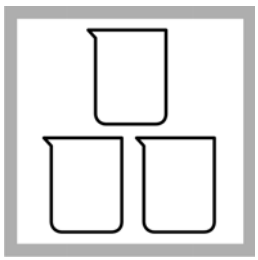
Sample pH measurement (calibration required)

1. Refer to the operation section of the electrode or meter manual to prepare the pH electrode and meter.

2. Connect the pH electrode to the meter.

3. Turn the meter on. Make sure that the meter is set to measure to measure pH.

4. For setup options such as measurement resolution, temperature units, calibration buffer set and other options refer to appropriate meter or electrode manual.



5. In three separate beakers or appropriate containers, prepare fresh buffers of 4.0, 7.0 and 10.0 pH.

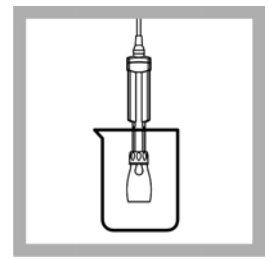
The sample pH should fall within the pH range of the calibration buffers. One, two or three calibration buffers may be used to calibrate.

Other pH calibration buffers sets may be used.

6. Calibrate the pH meter and electrode as directed in the instructions in the meter or probe manual.

Make sure that the calibration slope is acceptable (typically -58 ±3 mV per pH unit at 25 °C).

7. Rinse the electrode in deionized water and blot dry prior to sample measurement. Rinse the electrode between measurements to minimize contamination.

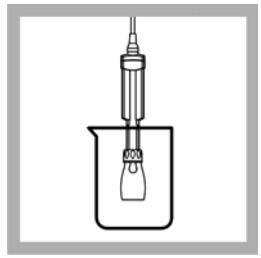


8. Put the electrode in the sample and press **READ**. For faster response, stir at a slow to moderate rate.

Sample pH measurement (calibration required) (continued)

9. When the measurement is stable, store or record the pH and temperature values.

For HQd meters, data is stored automatically when Press to Read or Interval is selected in the Setup Measurement Mode. When Continuous is selected, data will only be stored when the key under **STORE** is pressed. For sens***ion*** meters, the STORE key must be pressed.



10. Store the pH electrode in pH storage solution when not in use. See *Sample collection, preservation, general storage and cleaning* for more details.

Low Ionic Strength (LIS) or high purity water measurements

Low ionic strength solutions have very low buffering capacity and readily absorb carbon dioxide from the air. When a sample absorbs carbon dioxide from the atmosphere, carbonic acid forms. Carbonic acid decreases the sample pH and increases conductivity, causing inaccurate readings. One solution to this problem is to test the sample in a low volume, airtight sample chamber such as a Low Ionic Strength (LIS) Chamber. Use refillable or platinum series electrodes for measurement of pH in LIS or high purity waters.

Initial use

1. Before measuring an LIS sample, soak the electrode in a solution similar to the sample in ionic strength and pH for 10 to 15 minutes.
2. Rinse the electrode with deionized water from a wash bottle.
3. Blot excess liquid with a soft paper towel.
4. Put the electrode in the sample.

Between uses
Between uses, in intervals of up to a two hours, the electrode can be stored in the sample (if the sample is not an extreme pH), or in a neutral LIS solution such as tap water. Rinse the electrode before use to prevent sample contamination.

Important Note: *If pH electrodes are stored in LIS samples for a long period of time, the electrode life may be shortened.*

After measuring the LIS samples, put electrode back into the electrode storage solution or 3 M KCl.

Sample collection, preservation, general storage and cleaning

- Collect samples in clean plastic or glass bottles. Fill completely and cap tightly.
- Analyze samples immediately, preferably in the field.
- Storage of an electrode is based on how long the electrode will be stored, how quickly the electrode needs to be used and the type of sample being measured. For general storage, use the Hach storage solution or a 3 M Potasium Chloride (KCl) solution.
- A contaminated glass bulb or fouled electrode may cause slow response times. Do not clean the bulb too often because the bulb life may shorten.
- To clean an electrode with general contamination, immerse the electrode tip in 0.1 N hydrochloric acid (HCl). Then, immerse the electrode in 0.1 N sodium hydroxide (NaOH) and again in 0.1 N hydrochloric acid, each for a 2-minute period. Rinse with deionized water and soak in deionized water for at least 15 minutes.
- To clean an electrode contaminated with oils and fats, immerse the electrode tip in a detergent solution. Use a soft brush or ultrasonic bath if necessary. Avoid scratching the glass bulb.

Interferences

- Acid error is negligible.
- Sodium error, usually present in alkaline solutions, is low but increases at pH values higher than pH 11.

For more detailed information, refer to the meter or electrode manual.

Accuracy check

Check electrode response
An electrode is responding properly if its calibration slope meets the slope specifications of the electrode (typically -58 ±3 mV at 25 °C).

Check calibration accuracy
Return the electrode to a calibration buffer and measure the pH to test the system. Rinse and recondition the electrode before measuring subsequent samples.

Method performance

The accuracy of a pH measurement depends on many factors associated with the overall pH system, including the pH meter, choice of electrode and pH standards or buffers used during pH calibration. Refer to the appropriate electrode and meter manual to determine method performance.

Summary of method

pH is a measure of the hydrogen ion activity in a solution and is defined as:

–log10 aH+

Where
aH+ is the activity of the hydrogen ion.

A Combination pH Electrode responds to the hydrogen ion concentration (activity) by developing an electrical potential at the glass/liquid interface. At a constant temperature, this potential varies linearly with the pH of the solution being measured.

Water with relatively high conductivity typically has a fairly high buffer capacity. Slight pH changes due to absorption of carbon dioxide are usually not significant. If the sample conductivity is not known and high accuracy is desired, follow either the LIS or high purity water methods.

Consumables and replacement items

Required apparatus and reagents

Description	Quantity	Unit	Catalog number
HQ meters and probes (select one meter and probe combination)			
HQ40d meter	1	each	HQ40d53000000
HQ30d meter	1	each	HQ30d53000000
HQ11d meter	1	each	HQ11d53000000
pH Gel Probe, standard, with 1 m cable	1	each	PHC10101
pH Gel Probe, standard, with 3 m cable	1	each	PHC10103
pH Liquid Probe, standard, with 1 m cable	1	each	PHC30101
pH Liquid Probe, standard, with 3 m cable	1	each	PHC30103
pH Gel Probe, rugged, with 5 m cable	1	each	PHC10105
pH Gel Probe, rugged, with 10 m cable	1	each	PHC10110
pH Gel Probe, rugged, with 15 m cable	1	each	PHC10115
pH Gel Probe, rugged, with 30 m cable	1	each	PHC10130
sension **meters and probes (select one meter and probe combination)**			
*sens**ion*** 1	1	each	5170000
*sens**ion*** 3	1	each	5175000
Electrolyte cartridge, potassium chloride	1	2/pkg	2546902
Gel Filled pH electrode	1	each	5193500
Refillable pH electrode, platinum series electrode (5191000 as #1); flat Platinum series electrode (5195000 as #4)	1	each	5194000
For LIS and high purity water measurements			
Low Ionic Strength (LIS) Chamber	1	each	5189900

Recommended standards

Description	Unit	Catalog number
Hach Solutions[1]		
pH Color buffer solution kit (NIST), 500 mL, includes:	each	2947600
pH 4.01 +/- 0.02 pH buffer (NIST)	500 mL	2283449
pH 7.00 +/- 0.02 pH buffer (NIST)	500 mL	2283549
pH 10.01+/- 0.02 pH buffer (NIST)	500 mL	2283649
Powder pillows[1]		
pH 4.01 +/- 0.02 pH buffer powder pillow (NIST)	50/pkg	2226966
pH 7.00 +/- 0.02 pH buffer powder pillow (NIST)	50/pkg	2227066
pH 10.01+/- 0.02 pH buffer powder pillow (NIST)	50/pkg	2227166
Radiometer Analytical (IUPAC Series certified pH standards):		
pH 1.679 ± 0.010 at 25 °C	500 mL	S11M001
pH 4.005 ± 0.010 at 25 °C	500 mL	S11M002
pH 7.000 ± 0.010 at 25 °C	500 mL	S11M004
pH 10.012 ± 0.010 at 25 °C	500 mL	S11M007
pH buffer 1.09, technical	500 mL	S11M009
pH buffer 4.65, technical	500 mL	S11M010
pH buffer 9.23, technical	500 mL	S11M011
Refill Solution and Storage:		
pH Filling Solution (for PHC301), 3M KCl, saturated with AgCl	30 mL	2841700
pH Electrode Storage Solution	500 mL	2756549

[1] Larger quantities are available

Optional reagents and apparatus

Description	Unit	Catalog number
Sample bottle, general purpose with Screw-cap, polypropylene, 500-mL	each	2758101
Sample bottle, cleaned and certified, HDPE, suitable for EPA reporting, 500-mL	each	2758201
*sens**ion*** 2 meter	each	5172511
*sens**ion*** 4 meter	each	5177500

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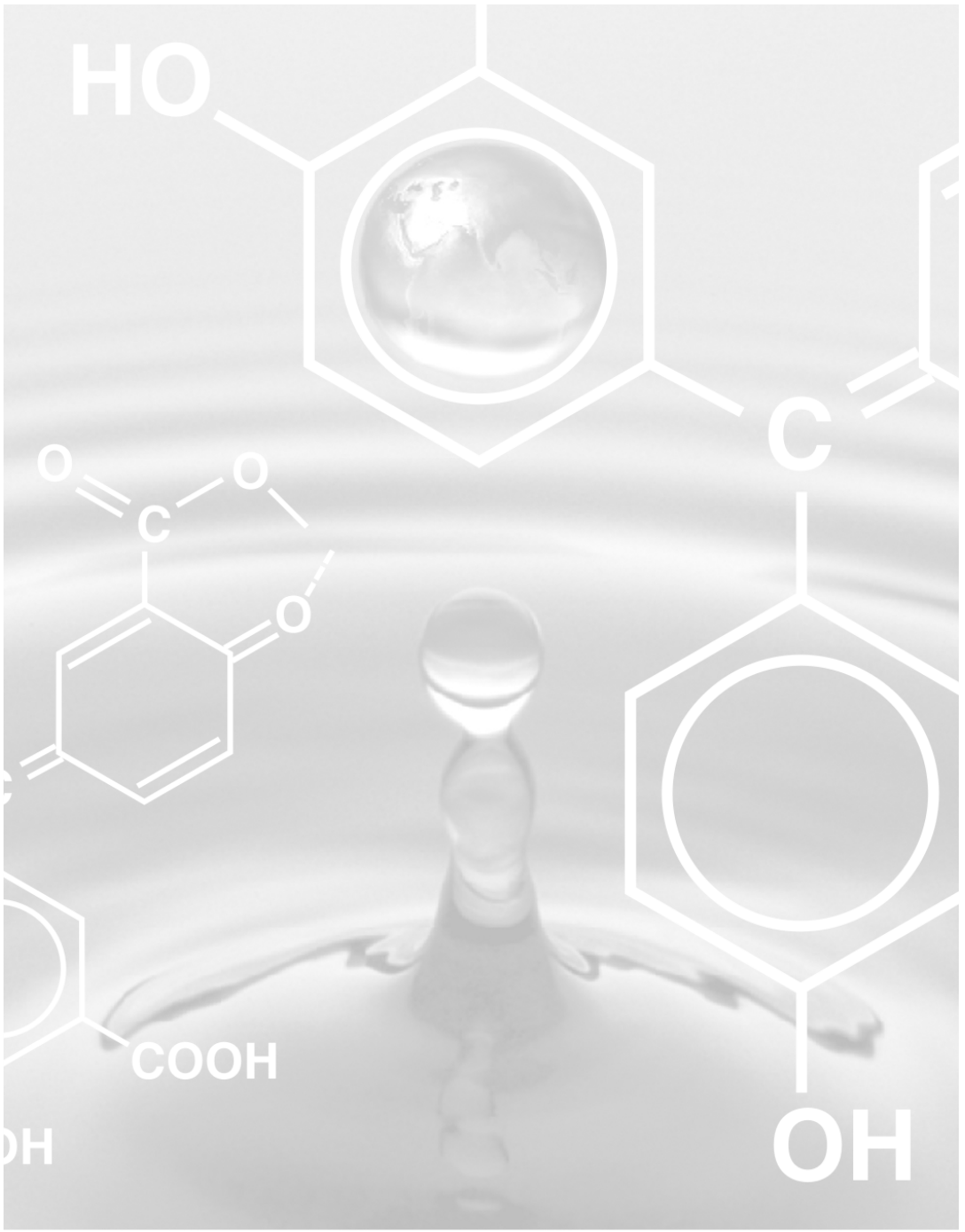
Edition 7


HO
O
O
C
O
C
COOH
OH
OH

Acidity

For water, wastewater and seawater **Digital Titration Method**

Acidity is the quantitative expression of water's capacity to neutralize a strong base to a designated pH and an indicator of how corrosive water is. Acidity can be caused by weak organic acids, such as acetic and tannic acids, and strong mineral acids including sulfuric and hydrochloric acids; however, the most common source of acidity in unpolluted water is carbon dioxide in the form of carbonic acid.

Acidity is classified by the pH value of a titration end point. Acidity caused by mineral acids exhibits a pH below 4.5. Salts of certain metals, particularly those with trivalent iron and aluminum, may hydrolyze in water and also contribute to acidity.

Standard Methods for the Examination of Water and Wastewater (*Standard Methods*) recommends titration with sodium hydroxide to an end point pH of 3.7 to determine mineral acidity. Titrate to pH 8.3 to determine total acidity.

Acidity is commonly determined using methyl orange as a color indicator of the pH end point. Because methyl orange undergoes a color change from red to orange at a pH of 3.7, the results of the titration are termed Methyl Orange Acidity. Hach procedures for acidity use bromphenol blue indicator instead of methyl orange because the methyl orange color change is difficult to detect. The bromphenol blue indicator gives a sharp end point change from yellow to blue-violet.

Total acidity includes acidity caused by mineral acids, weak organic acids, and carbon dioxide (in the form of carbonic acid). Acidity determined by titrating to a phenolphthalein end point pH of 8.3 corresponds to the neutralization of carbonic acid to bicarbonate. Because carbon dioxide is the major cause of acidity in natural waters, in most cases the phenolphthalein acidity is equal to the total acidity. Acidity tests can be performed using a pH meter to detect the end points; however, methyl orange acidity and phenolphthalein acidity are the terms used to describe the results. Results of the acidity tests are reported in mg/L $CaCO_3$.

Table 427 Chemical reactions

Methyl orange acidity	Phenolphthalein acidity
$2NaOH + H_2SO_4 \rightarrow Na_2SO_4$	$NaOH + H_2CO_3 \rightarrow NaHCO_3 + H_2O$
$NaOH + HCl \rightarrow NaCl + H_2O$	$NaOH + HC_2H_3O_2 \rightarrow NaC_2H_3O_2 + H_2O$

Reactions of indicator Phenolphthein

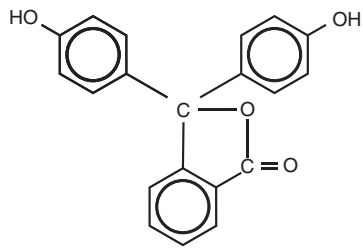


Figure 36 Colorless—pH < 8.3

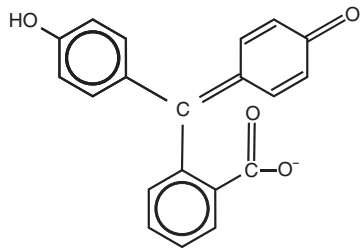


Figure 37 Pink—pH > 8.3

Reactions of indicator Bromphenol blue*

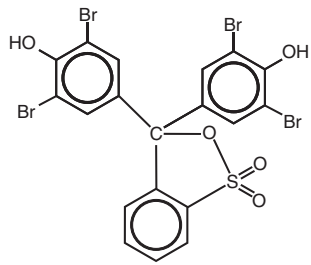


Figure 38 Yellow=pH 3

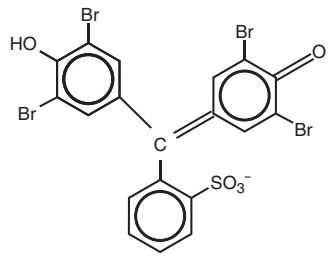


Figure 39 Blue-violet=pH 4.6

* 3',3",5',5", -Tetrabromophenolsulfonephthalein

Alkalinity

For water, wastewater and seawater **Titration Method**

Introduction

Alkalinity is a measure of the capacity of water to neutralize acids. Alkalinity of water is due primarily to the presence of bicarbonate, carbonate, and hydroxide ions. Salts of weak acids, such as borates, silicates and phosphates, may also contribute. Salts of certain organic acids may contribute to alkalinity in polluted or anaerobic water, but their contribution usually is negligible. Bicarbonate is the major form of alkalinity. Carbonates and hydroxide may be significant when algal activity is high and in certain industrial water and wastewater, such as boiler water.

Alkalinity is significant in the treatment processes for potable water and wastewater. The alkalinity acts as a pH buffer in coagulation and lime-soda softening of water. In wastewater treatment, alkalinity is an important parameter in determining the amenability of wastes to the treatment process and control of processes such as anaerobic digestion, where bicarbonate alkalinity, total alkalinity and any fraction contributed by volatile acid salts become considerations.

Alkalinity is expressed as phenolphthalein alkalinity or total alkalinity. Both types can be determined by titration with a standard sulfuric acid solution to an end point pH, evidenced by the color change of a standard indicator solution. The pH also can be determined with a pH meter.

Phenolphthalein alkalinity is determined by titration to a pH of 8.3 (the phenolphthalein end point) and registers the total hydroxide and one half the carbonate present. Total alkalinity is determined by titration to a pH of 4.9, 4.6, 4.5, or 4.3, depending on the amount of carbon dioxide present. The total alkalinity includes all carbonate, bicarbonate and hydroxide alkalinity.

The following end points are recommended for determining total alkalinity in water samples of various compositions and alkalinity concentrations.

Table 428 Determine total alkalinity

Sample Trait	End Point
Alkalinity approximately 30 mg/L	pH 4.9
Alkalinity approximately 150 mg/L	pH 4.6
Alkalinity approximately 500 mg/L	pH 4.3
Silicates or phosphates known present or suspected	pH 4.5
Industrial waste or complex system	pH 4.5
Routine or automated analyses	pH 4.5

Chemical reactions

Sulfuric acid (hydrochloric acid may be used) reacts with the three forms of alkalinity, converting them to water or carbonic acid. If hydroxide is present, it reacts to form water:

$$2OH^- + H_2SO_4 \rightarrow 2H_2O + SO_4^{2-}$$

This conversion usually is complete at a pH of about 10. Phenolphthalein alkalinity is determined by titration to an end point pH of 8.3, which corresponds to the conversion of carbonate to bicarbonate.

$$2CO_3^{2-} + H_2SO_4 \rightarrow 2HCO_3^- + SO_4^{2-}$$

If hydroxide is present, titration to pH 8.3 will indicate the alkalinity due to all of the hydroxide plus one-half of the carbonate. Continued titration to pH 4.5 completes the conversion of carbonate plus any bicarbonate present to carbonic acid. This value is termed Total Alkalinity.

$$2HCO_3^- + H_2SO_4 \rightarrow 2H_2CO_3 + SO_4^{2-}$$

Methyl red

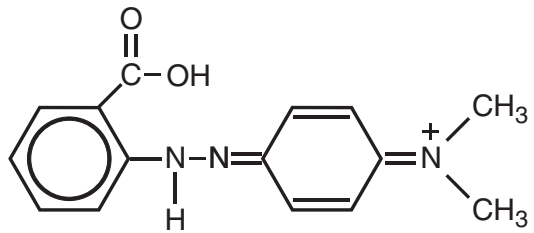


Figure 40 Red=pH 4.8

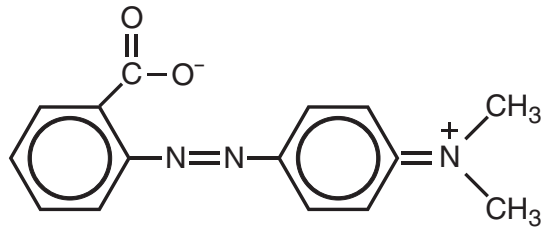


Figure 41 Yellow=pH 6.0

Bromcresol green

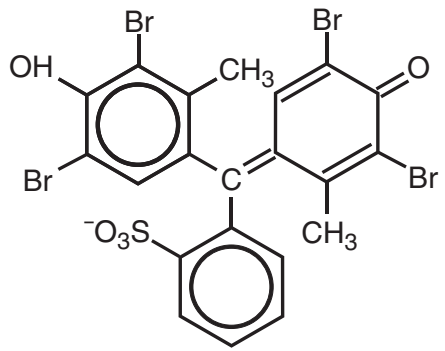


Figure 42 Blue=pH 5.5

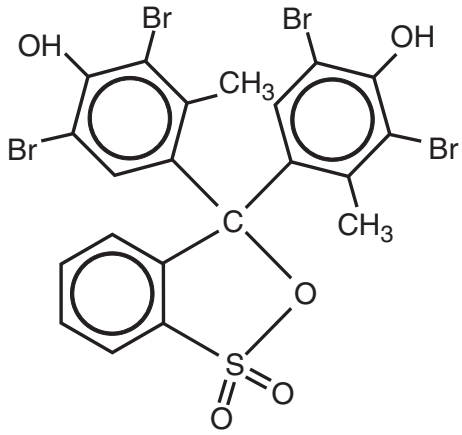


Figure 43 Yellow=pH 3.8

Aluminum

For water **Aluminon Method**

Introduction

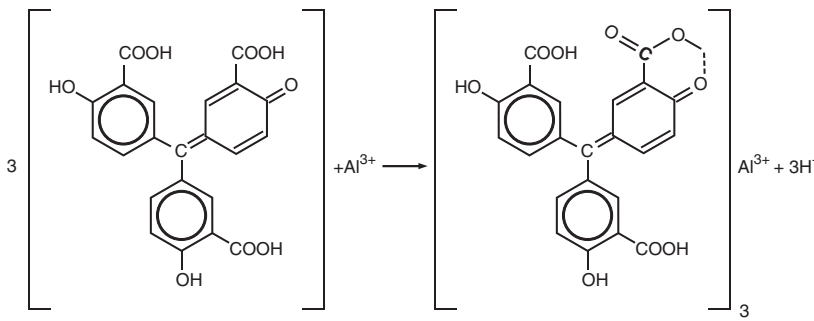
Aluminum, the earth's most abundant metal, is present in natural waters through contact with rocks, soil and clay. Alum coagulation in water clarification systems may also contribute to the aluminum content of treated water, although only 20–50 µg/L of aluminum remain in the finished product from a well-controlled operation.

The Aluminon Method is one of the oldest and most thoroughly documented methods available for determining aluminum in water. The AluVer 3™ Aluminum Reagent used in this method is packaged in powder pillow form, providing exceptional stability.

Chemical reactions

AluVer is an aluminon reagent in combination with a pH buffer. AluVer 3 reacts with aluminum present in a sample to form a reddish-colored solution in direct proportion to the aluminum concentration.

Ascorbic acid is added prior to the addition of AluVer 3 to eliminate interference due to iron. To establish a reagent blank, the sample is split after the addition of the AluVer 3. Bleaching 3 Reagent is then added to one-half of the split sample to bleach out the color of the aluminum aluminon complex.



Aurintricarboxylic acid

Figure 44 Chemical reaction

Barium

For water, wastewater, oil-field water and seawater

Turbidimetric Method

Introduction

Although barium is relatively abundant in nature, usually only trace amounts are found in water. Barium concentrations average about 0.05 mg/L in potable waters, but may range as high as 0.9 mg/L in some natural waters. More than 1 mg/L of barium implies that the water is not suitable for drinking and is polluted by industrial wastes. Barium and its compounds can be found in pigments, rat poisons, fireworks, and are used in rubber making, x-ray photography and even as weighting agents for oil well drilling.

Chemical reactions

Barium is determined by adding sulfate to the water sample to form barium sulfate, which precipitates. These particles are held in suspension as colloids by the BariVer™ 4 Reagent. The barium concentration is determined by measuring the resulting turbidity using a spectrophotometer or colorimeter. The barium concentration is proportional to the increase in turbidity when barium sulfate precipitates. The Hach procedure uses sodium sulfate, contained in BariVer 4 Reagent Powder, as the source of sulfate. The BariVer 4 Method is especially useful for brines where barium and sulfate coexist in solution and precipitation usually cannot be initiated by the simple addition of more sulfate.

$$Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4$$

Boron

For water and wastewater **Azomethine-H and Carmine Methods**

Introduction

Boron normally occurs in natural waters at concentrations less than 1.0 mg/L. Boron in natural water could be an indicator of sanitary pollution from domestic wastewater, usually in the form of borates from laundry detergents. In water for human consumption, boron concentrations typically should be less than 300 µg/L. Large amounts of boron can affect the central nervous system; when continually ingested over an extended period of time boron can cause a syndrome called borism.

In the semiconductor industry, boron has been used as an indicator of ion-exchange resin exhaustion in wafer rinsewater treatment. Boron is routinely monitored in irrigation water since many varieties of plants are sensitive to excess boron.

Analytical colorimetric methods for boron include the Curcumin Method, the Carmine Method and the Azomethine-H Method.

Chemical reactions

Azomethine-H method

The Azomethine-H Method involves the coupling of H-acid with an aromatic hydroxyaldehyde, such as salicylaldehyde, due to the catalytic effect when boron is present. At neutral pH values and a controlled temperature, the condensation reaction is completed quickly (within 15 minutes). After product formation, the solution is adjusted to an acidic pH for optimum color measurement at 410 nm (yellow) using a colorimeter or spectrophotometer. The method is sensitive and highly selective for the determination of dissolved boron in water.

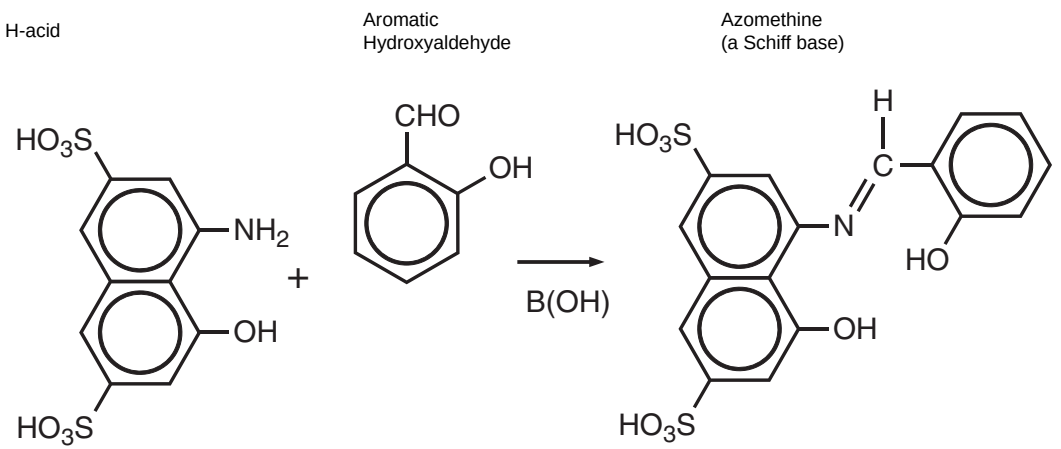
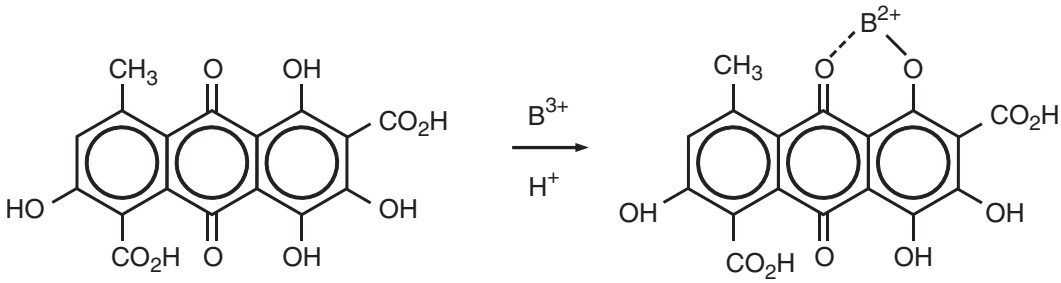


Figure 45 Chemical reactions for the Azomethine-H method

Carmine method

In the presence of concentrated sulfuric acid, boron exists as the cation B^{3+}. The cation complexes to the carmine indicator causing the solution to change color from red to blue.

The blue-colored complex is read at 605 nm using a spectrophotometer, and the amount of color is proportional to the dissolved boron concentration.



Carmine

Boron—Carmine Complex

Figure 46 Chemical reactions for the Carmine method

Benzotriazole and Tolyltriazole

For water **Ultraviolet Digestion Method**

Introduction

Benzotriazole and tolyltriazole (BZT and TTA) are used extensively as corrosion inhibitors for copper alloys. In both open and closed recirculating water cooling systems, concentrations of 2 to 10 mg/L provide effective corrosion control after initial passivation

Benzotriazole Tolyltriazole

Figure 47 Chemical procedures

The Hach method for BZT and TTA determinations offers substantial improvements over conventional analytical methods. Time-consuming conventional methods, such as ultraviolet (UV) spectroscopy, liquid chromatography and potentiometric titration, require expensive equipment and highly skilled personnel. The Hach method uses simple, inexpensive equipment and can be performed in less than 10 minutes without loss of accuracy or precision. The analysis range is 0–15 mg/L at a wavelength of 425 nm.

Chemical reactions

This method of analysis is based on the UV-photolysis of triazole in the presence of a chemical catalyst to form a dimer or polymer of the triazole. A stoichiometric amount of a soluble yellow-colored compound is then formed. The calibration follows Beer's Law throughout the 0–15 mg/L concentration range, with an estimated detection limit of approximately 0.3 mg/L

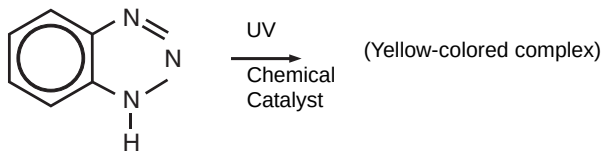


Figure 48 Chemical reaction

Carbon Dioxide

For water and seawater **Titration Method**

Introduction

Carbon dioxide is present in all surface waters, generally in amounts less than 10 mg/L; however, higher concentrations are not uncommon in ground waters. Dissolved carbon dioxide has no harmful physiological effect on humans and is used to recarbonate water during the final stages of water-softening processes and also to carbonate soft drinks. High concentrations of dissolved carbon dioxide are corrosive and have been known to kill fish.

The analysis for carbon dioxide is similar to that for acidity. A water sample is titrated to a phenolphthalein end point with Sodium Hydroxide Standard Solution. Strong mineral acids are assumed to be absent or to be negligible in effect. Care must be taken during the analysis to minimize the loss of carbon dioxide from the water sample as a result of aeration during collection and swirling of the sample.

Chemical reactions

The reaction of sodium hydroxide with carbon dioxide (as carbonic acid) occurs essentially in two steps; first a reaction from carbonic acid to bicarbonate and then to carbonate.

Because the conversion of carbon dioxide to bicarbonate is complete at pH 8.3, phenolphthalein can be used as a color indicator for the titration. The sodium hydroxide titrant must be of high quality and be free from sodium carbonate.

$CO_2 + H_2O \rightarrow H_2CO_3$ (Carbonic acid)

$H_2CO_3 + NaOH \rightarrow NaHCO_3 + H_2O$

$NaHCO_3 + NaOH \rightarrow Na_2CO_3 + H_2O$

Chloramine (Mono)

For water and wastewater **Indophenol Method**

Introduction

Chloramination disinfection is the practice of forming inorganic chloramines in water to reduce microbial concentrations to within acceptable limits. The chloramines; monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and nitrogen trichloride (NCl_3), form when chlorine and ammonia are combined in water. Traditionally, treated wastewater, which contains ammonia, is disinfected by the addition of chlorine. In recent years, many drinking water facilities have converted to chloramination to disinfect potable water. Roughly 20% of all drinking water facilities in the United States now use chloramines as the residual disinfectant.

For the chloramination of drinking water, monochloramine is the preferred disinfectant. Formation of dichloramine and nitrogen trichloride is avoided, since more chlorine is consumed and the presence of these chloramines can produce odors or off-tastes.

In treated wastewater, any organic nitrogen compounds present will form organic chloramines during chlorination. Organic chloramines, as a class, are much weaker disinfectants than the inorganic chloramines. Chlorine overfeeds and ineffective mixing can lead to greater production of organic chloramines, thereby diminishing the total germicidal activity.

Hach chemists have developed a method for the specific determination of monochloramine in water. The method is based on the classic indophenol chemistry for determining ammonia. The chemistry has been improved to increase the specificity of the method for inorganic monochloramine in the presence of organic chloramines. In addition, the method was modified to greatly accelerate the color development time and increase the precision of the test. The new test has been shown to be specific for monochloramine, without interference from organic or inorganic amines, dichloramines, free chlorine, organic chloramines, nitrites or manganese.

Chemical reactions

Monochloramine reacts specifically with a substituted phenate to form a quinone imine intermediate. In the presence of a cyanoferrate, the intermediate couples with excess phenate to form a green-colored indophenol. The amount of indophenol formed is proportional to concentration of monochloramine in the sample. See the *Chemical reactions* figure below.

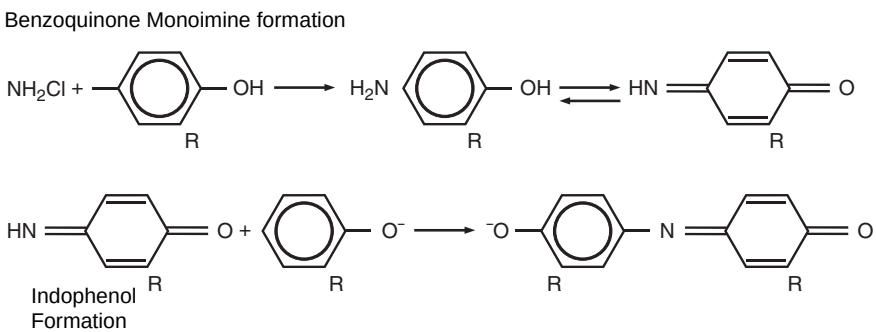


Figure 49 Chemical reactions[1]

[1] R= reaction accelerating group

Chloramine (Mono); Nitrogen, Free Ammonia

Indophenol method

For determining free ammonia and monochloramine simultaneously in finished chloraminated water

Introduction

Chloramination disinfection is the practice of forming inorganic chloramines in water to reduce microbial concentrations to within acceptable limits. The chloramines—monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3)—form when chlorine and ammonia are combined in water. In recent years, many drinking water facilities have converted from free chlorination to chloramination to disinfect potable water. Chloramines are weaker oxidants than free chlorine and therefore minimize the formation of harmful disinfection by-products.

A typical chloramination curve is presented in Figure 50. For the chloramination of drinking water, monochloramine is the preferred disinfectant (Section I of the curve). This is optimized by an approximate 5:1 ratio (by weight) of chlorine to ammonia. Adding too much chlorine leads to the decrease of monochloramine and the formation of dichloramine and trichloramine, causing taste and odor problems (Section II). Adding too little chlorine leaves excess unreacted or "free" ammonia in the water which acts as a food source and can lead to nitrification and bacterial growth in the distribution system. At the breakpoint, which is the vertical line between Sections II and III, no monochloramine remains. Any additional chlorine added will be in the form of free chlorine.

In treated wastewater, any organic nitrogen compounds present will form organic chloramines during chlorination. Organic chloramines, as a class, are much weaker disinfectants than the inorganic chloramines. Chlorine overfeeds and ineffective mixing can lead to greater production of organic chloramines, thus diminishing the total germicidal activity.

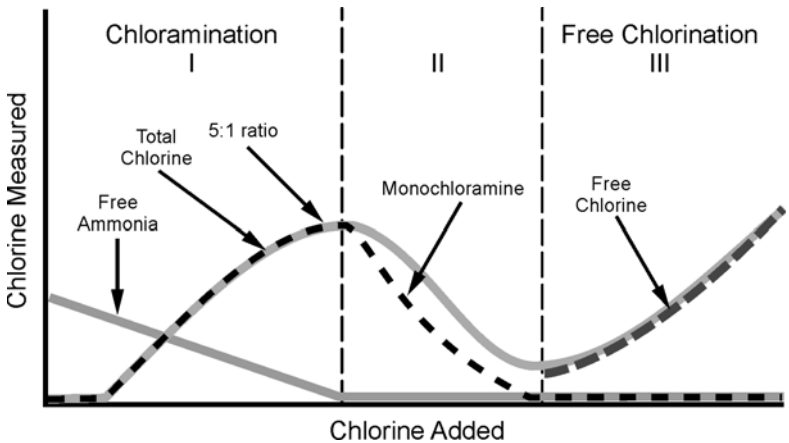


Figure 50 Breakpoint curve for chloraminated water

Chemical reactions

Refer to Figure 51 for the indophenol method mechanism.

Added hypochlorite combines with free ammonia to form more monochloramine (1). In the presence of a cyanoferrate catalyst, monochloramine in the sample reacts with a substituted phenol to form an intermediate monoimine compound (2). The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample (3). Free ammonia is determined by comparing the color intensities, with and without added hypochlorite.

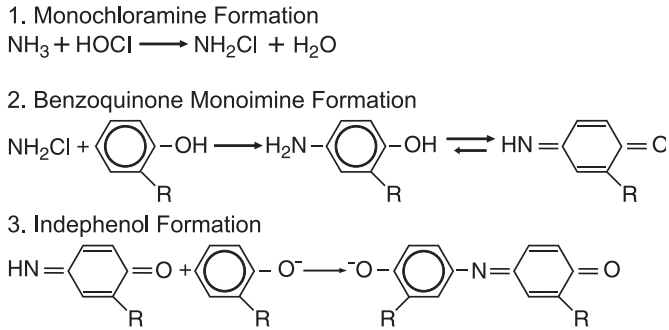


Figure 51 Chemical reactions for the indophenol method

Chloride

For water and wastewater

Mercuric Nitrate, Mohr Argentometric and Mercuric Thiocyanate Methods

Introduction

Chlorides are present in all potable water supplies and in sewage, usually as a metallic salt. When sodium is present in drinking water, chloride concentrations in excess of 250 mg/L give a salty taste. If the chloride is present as a calcium or magnesium salt, the taste detection level may be as high as 1000 mg/L chloride.

Chloride is essential in the human diet and passes through the digestive system unchanged, thereby becoming one of the major components of raw sewage. The wide use of zeolite spheres in water softeners also contributes a large amount of chloride to sewage and wastewaters.

High chloride concentrations in water are not known to have toxic effects on humans, although large amounts may act corrosively on metal pipes and be harmful to plant life. The maximum allowable chloride concentration of 250 mg/L in drinking water has been established for reasons of taste rather than as a safeguard against physical hazard.

Chemical reactions

Mercuric nitrate method
Mercuric nitrate reacts selectively with all the chloride present in a sample to produce mercuric chloride and nitrate ions. When all the chloride present in the sample has been complexed, excess mercuric ions combine with diphenylcarbazone to form a purple-colored complex indicating the end point. Hach procedures use Diphenylcarbazone Reagent Powder containing the indicator and a buffer for maximum convenience and reagent stability.

$$Hg(NO_3)_2 + 2Cl^- \rightarrow HgCl_2 + 2NO_3^-$$

Silver nitrate method (Mohr Argentometric method)
In the chloride test, using silver nitrate as the titrant and potassium chromate as the indicator, silver nitrate first reacts selectively with the chloride present in the sample to produce insoluble white silver chloride. After all the chloride has been precipitated, the silver nitrate then reacts with the potassium chromate to form an orange-colored silver chromate precipitate, thereby marking the end point of the titration. Potassium chromate indicator is combined with a buffer in Chloride 2 Indicator Powder.

$$AgNO_3 + K_2CrO_4 + Cl^- \rightarrow AgCl + NO_3^- + K_2CrO_4$$

$$2AgNO_3 + K_2CrO_4 \rightarrow \underset{\text{(Orange)}}{Ag_2CrO_4} + 2KNO_3$$

Mercuric Thiocyanate method

Colorimetric determination of chloride by the Mercuric Thiocyanate Method involves reaction of chloride in the sample with mercuric thiocyanate to produce mercuric chloride and free thiocyanate ions. In the presence of Fe^{3+} (ferric ion), the free thiocyanate ion forms highly colored ferric thiocyanate in proportion to the chloride concentration. Two liquid reagents have been formulated for this test: Mercuric Thiocyanate Solution and Ferric Ion Solution.

1. $\underset{\text{(Mercuric Thiocyanate)}}{Hg(SCN)_2} + 2Cl^- \rightarrow HgCl_2 + 2SCN^-$

2. $Fe^{3+} + 3SCN^- \rightarrow \underset{\text{(Red-orange)}}{Fe(SCN)_3}$

Chlorine Dioxide

For water and wastewater **DPD and Chlorophenol Red Methods**

Introduction

Chlorine Dioxide is a deep yellow gas that is generated directly for on-site use as a bleaching agent in industrial processes, such as the manufacture of pulp and paper. It is used increasingly for special treatment objectives in municipal water treatment because, unlike chlorine, chlorine dioxide does not form trihalomethanes (THMs) in reaction with certain organic compounds. Two colorimetric methods for chlorine dioxide at low levels are used in Hach procedures. Hach also offers a high range method that directly measures the yellow color of the chlorine dioxide gas dissolved in the sample water.

The DPD method is an extension of the N,N-diethyl-p-phenylenediamine (DPD) method for determining free and total chlorine. Glycine is used to eliminate chlorine interference.

The Chlorophenol Red (CPR) method reacts specifically with chlorine dioxide.

Chemical reactions

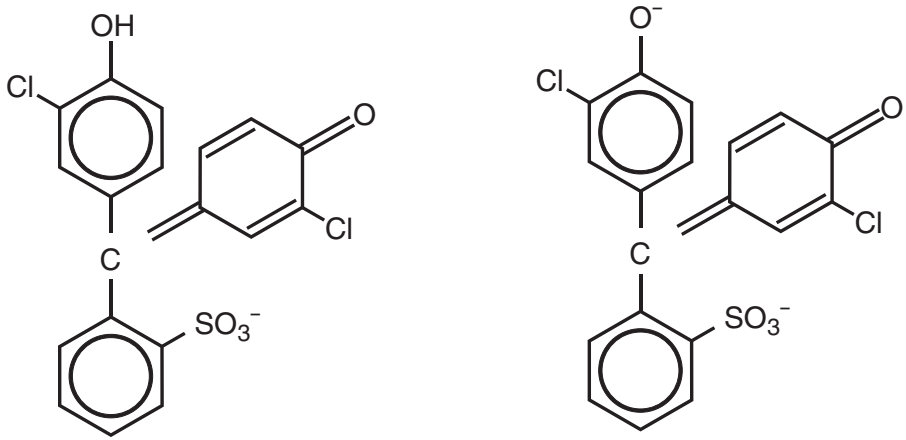
DPD Method

Chlorine dioxide reacts with the DPD (N,N-diethyl-p-phenylenediamine) Indicator Reagent (to the extent of one-fifth of its total available chlorine content corresponding to the reduction of chlorine dioxide to chlorite) to form a pink color. The color intensity is proportional to the ClO_2 in the sample. Chlorine interference is eliminated by adding glycine, which converts free chlorine to chloroaminoascorbic acid, but has no effect on chlorine dioxide at the test pH.

Chlorophenol red method

Chlorophenol Red (CPR) indicator reacts specifically with chlorine dioxide with a distinct color change; no interference is experienced form other mild oxidants, including hypochlorite, chlorite, chromate, permanganate, ferric iron, or low levels of chloramines. One mole of CPR reacts with two moles of chlorine dioxide to form a colorless product with a net decrease in absorbance at 570 nm. The discoloration of CPR is linear to approximately 0.6 mg/L, although concentrations to approximately 1.0 mg/L are easily determined. The reaction of CPR with ClO_2 is reproducible. No equation for this reaction will be suggested; however, the reaction may result in the formation of an ion-pair complex.

The reaction of CPR with chlorine dioxide is pH-sensitive. A pH of 7.0 has been suggested for the spectrophotometric method. Hach researchers found the optimum pH for this reaction is actually 5.2. It was also determined that the sensitivity is improved if the solution is buffered to near pH 10 after the initial reaction. The reagents for this method are contained in three convenient solutions. Reagent 1 is a buffer which adjusts the sample to the optimum pH, 5.2. Reagent 2 is a special formulation of CPR which is added after the pH adjustment. Reagent 3 is a pH 10 buffer added after CPR to increase sensitivity. Blanks for standardizing the spectrophotometer are prepared by adding dechlorinating agent to a 50-mL sample, thereby destroying up to 35 mg/L of ClO_2.



Yellow (acid color) Red (base color)

Figure 52 Chlorophenol Red structures

Chlorine, Free and Total

For water, wastewater and seawater **DPD Method**

Introduction

Chlorine is the disinfectant most frequently used for water and wastewater treatment. It was used first for industrial applications and to control odor in wastewater, in the early 1800s. The subsequent use of chlorine to disinfect water occurred by the mid-1800s. Industrial uses of chlorine include applications such as bleaching paper and controlling nuisance organisms in cooling towers.

Hydrochloric and hypochlorous acids are formed when chlorine is added to water. The disinfectant and form causing bleaching action, is hypochlorous acid.

$$Cl_2 + H_2O \rightarrow HCl + HOCl \text{ (Hypochlorous acid)}$$

Depending upon variables such as pH, temperature and the amount of organic or ammonia nitrogen, other forms of chlorine in water may include hypochlorite ions (OCl^-) and chloramines. Chlorine existing in water as hypochlorous acid or the hypochlorite ion is termed *free available chlorine*. Chloramines, including monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and nitrogen trichloride (NCl_3) are referred to as Combined Available Chlorine. Total Chlorine refers to the sum of free and combined available forms.

Methods for determining free, combined and total chlorine include: amperometric titration, colorimetric DPD, titrimetric DPD and iodimetric titration. The most widely used method, colorimetric DPD, is easy to perform, requires little apparatus, is inexpensive and adapts well to field test situations. DPD (N,N-diethyl-p-phenylenediamine) is oxidized by chlorine, causing a magenta (red) color. The intensity of color is directly proportional to the chlorine concentration. DPD reacts in much the same way with other oxidants, including bromine, chlorine dioxide, hydrogen peroxide, iodine, ozone and permanganate.

Chemical reactions

Free available chlorine
Hypochlorous acid and the hypochlorite ion oxidize DPD causing a magenta color. The reaction is pH dependent. DPD and appropriate buffer are packaged together in DPD Free Chlorine Reagent Powder Pillows to handle high levels of hardness without precipitation.

Total chlorine
Potassium iodide is added to the reaction to determine combined available chlorine forms and total chlorine. Chloramines oxidize the iodide to iodine; then the liberated iodine reacts with DPD to form the magenta color. DPD Total Chlorine Reagent Powder Pillows from Hach contain DPD, potassium iodide and a buffer.

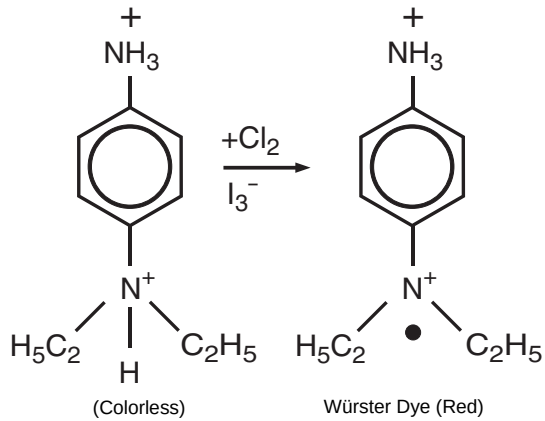


Figure 53 DPD chemical reactions

Chromium

For water and wastewater **Total and Hexavalent Methods**

Introduction

Chromium may be present in water as the hexavalent (chromate) or the trivalent form, although trivalent chromium rarely occurs in potable water. Hexavalent chromium enters a water supply through industrial wastes from metal plating baths and from industrial cooling towers where chromate is used to inhibit metal corrosion. Chromium is an objectionable contaminant in public drinking water supplies due to its suspected carcinogenic effects. Chromium present in potable waters above a 3-µg/L level indicates the possible presence of industrial wastes. Concentrations greater than 50 µg/L are sufficient grounds to reject the water supply.

Chemical reactions

Hexavalent chromium

Hexavalent chromium is determined by the 1,5-Diphenylcarbohydrazide Method using a single dry powder formulation called ChromaVer 3™ Chromium Reagent. This reagent contains an acidic buffer combined with 1,5-Diphenylcarbohydrazide which reacts to give a purple color when hexavalent chromium is present. The method is applicable to fresh water and wastewater samples. Color development is directly proportional to the amount of hexavalent chromium present.

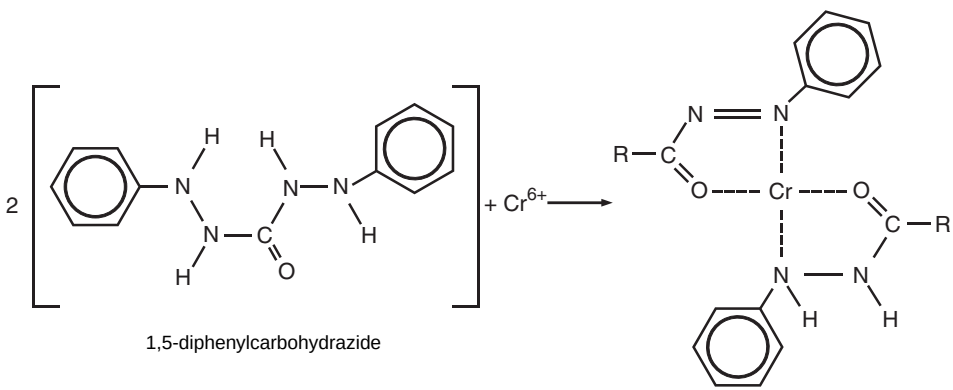


Figure 54 Hexavalent chromium chemical reaction

Total chromium

In the analysis for total chromium, the sample is heated to the boiling point under strong alkaline conditions in the presence of hypobromite. The trivalent chromium is converted to hexavalent chromium. The proper chemical conditions for this oxidation are provided by Chromium 1 Reagent Powder.

After the oxidation is complete, excess hypobromite is destroyed by the addition of Chromium 2 Reagent Powder. Then ChromaVer 3 Chromium Reagent, which contains an acidic buffer combined with 1,5-Diphenylcarbohydrazide, is added. A purple color develops with an intensity directly proportional to the total chromium concentration. The trivalent chromium can be determined by subtracting the hexavalent chromium test results from the results obtained in the total chromium test.

$$2Cr^{3+} + 3\ OBr^{-} + 10\ OH^{-} \rightarrow 2CrO_4^{2-} + 3Br^{-} + 5H_2O$$

Cobalt

For water **1-(2-Pyridylazo)-2-Naphthol (PAN) Method**

Introduction

Cobalt is valuable because of its ability to increase the strength and corrosion resistance of alloys. It is associated with nickel, silver, lead, copper and iron ores, from which it is most frequently obtained as a by-product. Cobalt is often found in industrial wastewaters as a corrosion product of alloys of iron, nickel and cobalt, but it seldom occurs in natural waters.

Toxicity of cobalt to aquatic life varies depending on pH, the species or organism, and synergetic effects. It is considered to be relatively nontoxic to humans. Methods for detection of low levels of cobalt historically have been limited to expensive and time-consuming techniques—mainly atomic absorption. By comparison, cobalt can be determined quantitatively by a simple colorimetric procedure using a spectrophotometer. Accuracy and precision rivals atomic absorption measurements. The very sensitive 1-(2-Pyridylazo)-2-Naphthol (PAN) Method is capable of detecting 0.1 mg/L cobalt. This unique method is relatively free from interferences and provides for simultaneous determinations of nickel and cobalt on the same sample portion without special treatments.

Chemical reactions

PAN is suspended in water by use of surfactants to allow it to form complexes with the metals in the sample. A complexing agent can be used to decompose all PAN chelates except those of cobalt, nickel and iron. A pH adjustment using the Phthalate-Phosphate Reagent aids in the masking of iron up to 10 mg/L, and also enhances the rate of development of the colored cobalt and nickel PAN complexes.

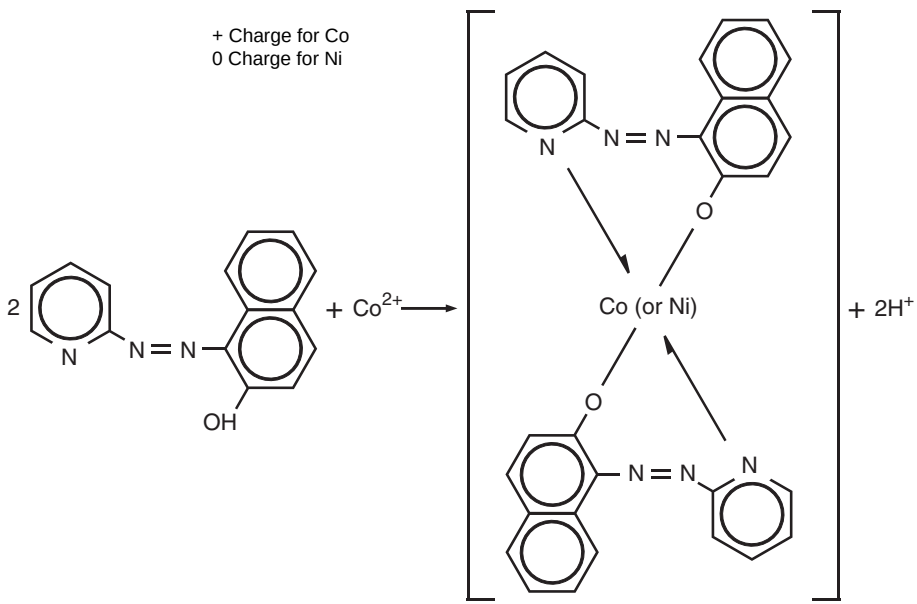


Figure 55 Formation of Co-PAN or Ni-PAN complex[1]

[1] Cheng, K. L., and Bray, R. H., Journal of Analytical Chemistry, 27, 1955, page 783.

The absorbance of the cobalt PAN complex at 560 nm is the same as at 620 nm; however, absorbance caused by the nickel PAN complex is zero at 620 nm. This difference in absorbance wavelengths allows cobalt to be determined without interference from nickel at a wavelength of 620 nm. Therefore, the nickel can be determined on the same sample by measuring the absorbance at 560 nm and subtracting the absorbance at 620 nm.

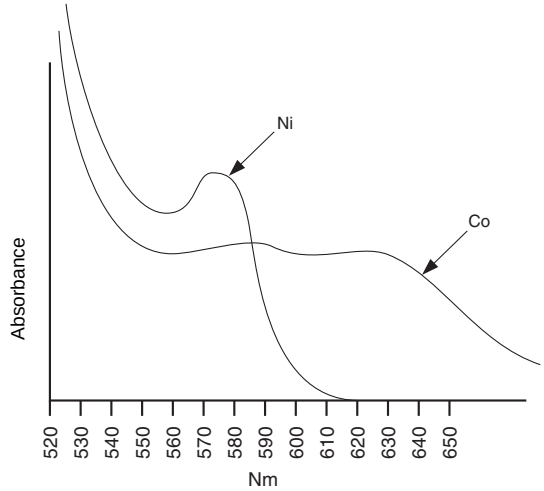


Figure 56 Typical absorbance scan for Co and Ni

Copper

For water, wastewater and seawater

Bicinchoninate, Porphyrin and Bathocuproine Methods

Introduction

Although copper comprises only 0.007% of the earth's crust, it is a very important element. Copper occurs in both free and combined forms throughout nature in many minerals. Copper may occur in natural waters, wastewaters, and industrial waste streams as soluble copper salts or as copper compounds precipitated on suspended solids. Forms of copper in water can be classified as *insoluble*, *dissolved* (free and complexed), and *total recoverable*. Insoluble copper includes precipitates such as copper sulfides and hydroxides. All copper in solution is known as dissolved copper, including Cu^{1+} (cuprous) and Cu^{2+} (cupric) ions and copper chelates such as CuEDTA.

Copper concentrations in potable water are usually very low. Copper is not considered a health hazard to humans although more than 1 mg/L can impart a bitter taste to water and large oral doses can cause vomiting and may eventually cause liver damage. Copper salts, such as copper sulfate ($CuSO_4$), may be used to control algae; however, they may also be toxic to fish and wildlife. Hach's simplified test procedures for copper use a variety of reagents to satisfy the desired range of detection and the form of copper to be measured. Hach procedures use primarily the Bicinchoninate and the Porphyrin Methods.

The *Copper reagents and applications* table lists proprietary reagents and applications.

Table 429 Copper reagents and applications

Reagent	Form measured		Application
	Without pretreatment	With digestion	
CuVer 1™[1]	Free	Total recoverable	water, wastewater
CuVer 2™	Total dissolved copper	Total recoverable	
Free copper reagent	Free	Total recoverable	hard water, wastewater, seawater
Porphyrin reagents	Free	Total recoverable	extremely low levels in water, wastewater and seawater

[1] CuVer is a trademark of Hach Company.

Chemical reactions

Bicinchoninate method

Copper can be determined by the reaction of copper with 2, 2'-biquinoline-4,4'-dicarboxylic acid (bicinchoninic acid). Bicinchoninate reacts with Cu^{1+} to produce a purple-colored complex.

Bicinchoninate does not react readily with Cu^{2+}. Determination of Cu^{2+} begins by reducing it to Cu^{1+}. The CuVer 1 Reagent combines the bicinchoninate reagent with a buffer and reducing agent, allowing determination of Cu^{1+} and Cu^{2+}. Total recoverable copper can be determined with this method if the sample is first digested to convert all of the copper present (including insoluble forms and complexed forms) to free copper.

Complexed copper forms such as CuEDTA react directly with CuVer 2™. Digestion is not necessary, and high levels of hardness do not interfere. The results will be in terms of total dissolved copper (free and complexed). When using CuVer 1, digestion is necessary and high levels of hardness interfere.

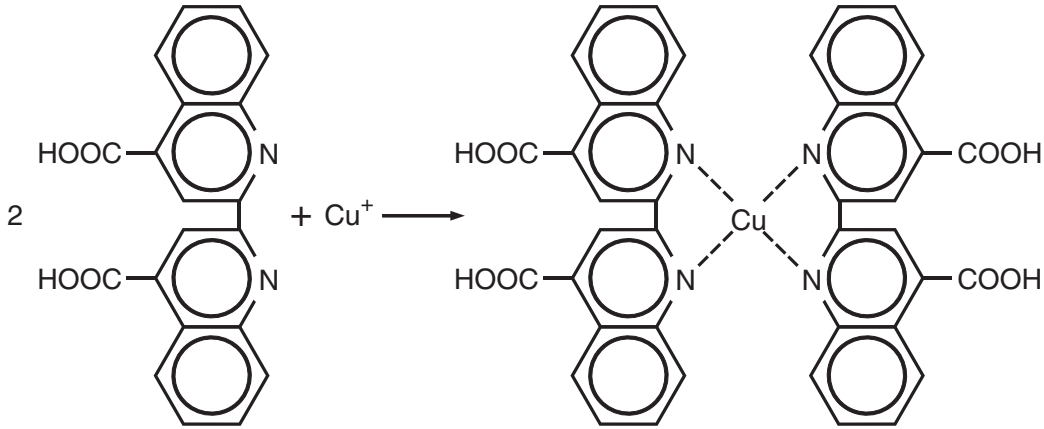


Figure 57 Reaction of Cu^{1+} and bicinchoninic acid

Use Free Copper Reagent Powder Pillows to determine free copper separately from complexed copper. The powder pillows contain bicinchoninate, a reducing agent and an inhibitor to eliminate calcium and magnesium interference. The results will be in terms of free copper. Complexed copper may then be determined by adding Hydrosulfite Reagent, repeating the analysis and subtracting the results of the two analyses.

Porphyrin method

The porphyrin method for determining copper is a very sensitive test, capable of detecting free copper (Cu^{1+} and Cu^{2+}) and total recoverable copper (with digestion) in the range of 0–150 µg/L.

Because of the sensitivity of the method, it is difficult to obtain water of high enough quality to establish a blank value. The porphyrin method uses a split sample. One half of the split sample is treated with a masking agent to complex the free copper forms; then, porphyrin reagent, a buffer, and a reducing agent is added. This forms a “zero blank” without the need for special copper-free water. Porphyrin reagent is added to the second half of the split sample, where it reacts with the free copper.

Interference caused by the reaction of porphyrin with other metals is minimized by using the split sample because interferences are compensated for in the blank. Porphyrin reacts slowly with Cu^{2+}, but a special formulation of the porphyrin and addition of a buffer allow the reaction with free copper to be completed within seconds. A reducing agent is also added in order to destroy unreacted porphyrin (which would otherwise interfere). An intense absorbance at 425 nm makes this method very sensitive when using a colorimeter or spectrophotometer. However, strong visual color development does not occur.

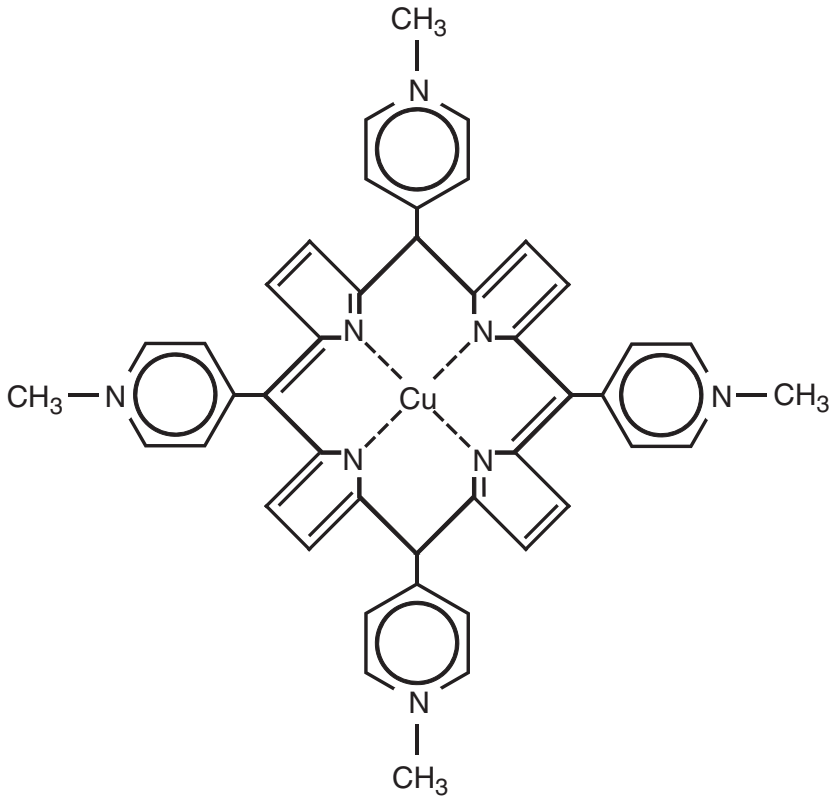


Figure 58 Final structure from the porphyrin method

Cyanide

For water, wastewater and seawater **Pyridine-Pyrazolone Method**

Introduction

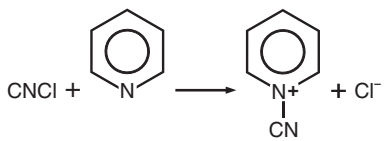
Cyanide is extremely toxic and occurs primarily in industrial effluents. Metal-cleaning and electroplating baths, gas scrubbers, gas works, coke ovens and other chemical treatments are the main sources of the cyanide found in industrial wastes. Natural waters do not contain cyanide; its presence usually indicates contamination from an industrial source. Proper neutral or alkaline chlorination of cyanide-containing wastewaters will reduce the level well below toxic limits.

Chemical reactions

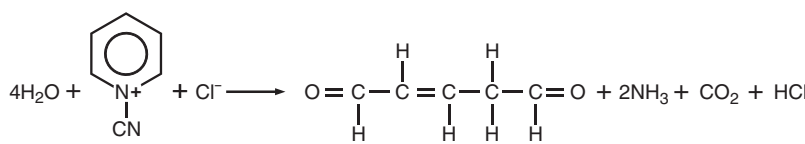
The cyanide test involves the following 4 steps:

(1) $2CN^- + Cl_2 \longrightarrow 2CNCl$

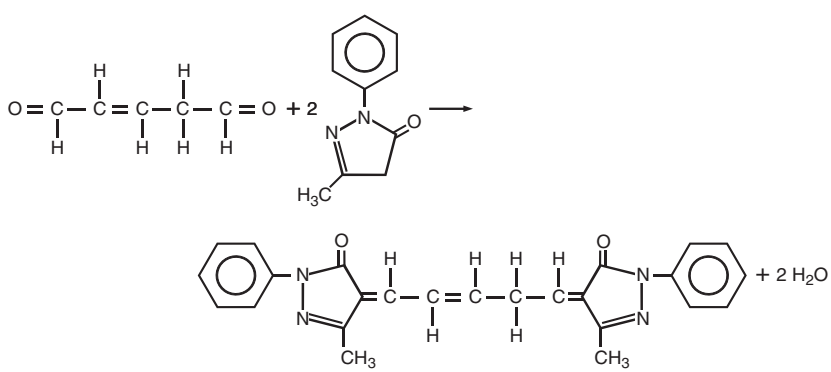
Cyanide is reacted with chlorine to produce cyanogen chloride (CNCl); the chlorine is provided by CyaniVer™ 3 Reagent.

(2) 

An intermediate nitrile is then formed by the addition of pyridine; the pyridine is provided by the addition of CyaniVer 4 Reagent. Excess chlorine is destroyed at this point.

(3) 

The nitrile is hydrolyzed to glutaconaldehyde; the reagent is provided in the CyaniVer 4 Reagent from the previous step.

(4) 

Finally, CyaniVer 5 Reagent, containing an excess of pyralozone, is added. The reaction with the glutaconaldehyde results in a blue color. The intensity of the color is directly proportional to the amount of cyanide present in the sample.

Fluoride

For water and seawater

SPADNS, SPADNS 2 and Ion-selective Electrode Methods

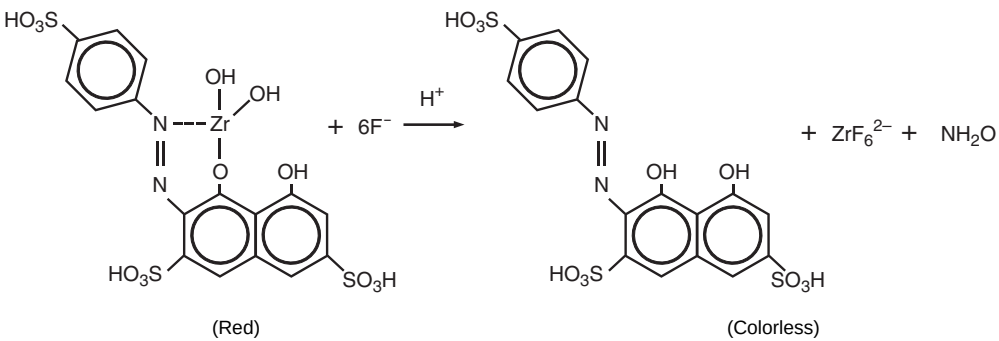
Introduction

Fluoride occurs naturally in some ground waters, and a 1-mg/L level is normally maintained in public drinking water supplies for the prevention of dental cavities. Excessive amounts of fluoride cause an objectionable discoloration of tooth enamel called “mottling”. For this reason, a permissible level in drinking water has been established by the USEPA in accordance with the Safe Drinking Water Act.

Chemical reactions

SPADNS method

The fluoride analysis involves the reaction of fluoride with a dark red zirconium-dye complex. Fluoride combines with part of the zirconium to form a colorless zirconium-fluoride complex with the net effect of bleaching the color. Measurement of the decrease in color intensity provides an accurate determination of the fluoride concentration. The SPADNS Method is the preferable colorimetric method due to its rapid reaction with fluoride and the stability of the SPADNS reagent.



(Red) (Colorless)

Figure 59 Chemical reaction for SPADNS method

SPADNS 2 method

Sodium Arsenite is used in the SPADNS method as a reducing agent to prevent interference from chlorine and other oxidants that are typically present in drinking water. The SPADNS 2 test eliminates arsenic from the original SPADNS formulation by using a non-toxic proprietary reducing agent to achieve identical results and test performance. All other chemistry remains the same as the SPADNS method.

Method of analysis

Ion-Selective electrode method

The Ion-Selective electrode method requires a Hach sens***ion***™ ISE Meter and an electrode system consisting of a silver/silver chloride reference electrode and a standard fluoride ion-selective electrode. Fluoride measurement is accomplished when a voltage potential is established across the lanthanum fluoride crystal on the end of the electrode; this potential is in direct proportion to the fluoride concentration of the sample. The meter is calibrated with fluoride standards bracketing the expected range. The concentration may be read directly from the meter. A total ionic strength adjustment buffer (TISAB) is used to eliminate interferences in the test, to adjust the pH to an optimum value and to introduce sufficient sodium chloride to mask variations in

ionic strength. TISAB reagent uses sodium 1,2-cyclohexanediaminetetraacetic acid (CDTA) for chelation of interfering metals, such as Al^{3+} and Fe^{3+}, as well as other complexing and buffering agents.

Formaldehyde

For water **MBTH Method**

Introduction

Formaldehyde is used in the treatment of fabric in textile industries, in metal plating baths, as a preservative for biological tissues and as a disinfectant in dialysis and reverse osmosis equipment.

The MBTH Method is a sensitive colorimetric test for low range measurement of aldehydes; it is most sensitive for formaldehyde.

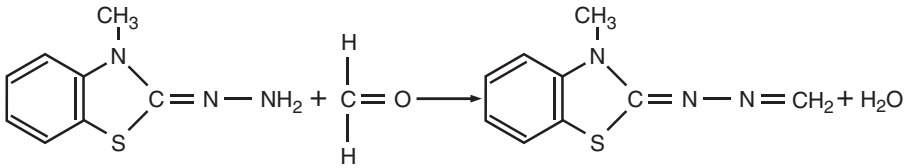
Chemical reactions

MBTH Method

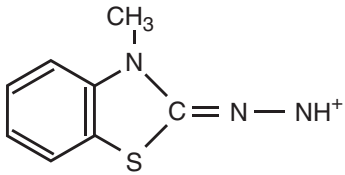
MBTH (3-methyl-2-benzothiazoline hydrazone) is added in excess to a sample containing formaldehyde, triggering multiple reactions. First, MBTH and formaldehyde react to form an azine (1). Excess MBTH is oxidized by addition of a developing solution (2). Oxidized MBTH reacts with the azine to form a species with an intense blue color (3). Intensity of the blue color is proportional to the original concentration of formaldehyde.

MBTH is contained in MBTH Powder Pillows. Liquid reagent for oxidizing excess MBTH is contained in the Developing Solution for Low Range Formaldehyde.

(1)

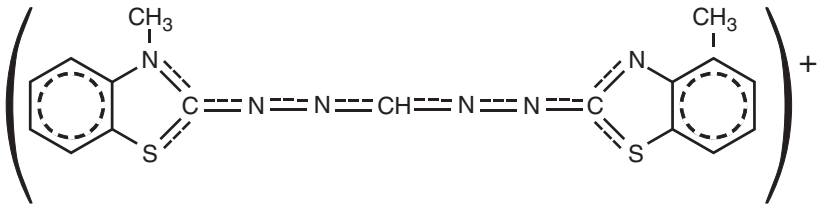


(2)



(Oxidized MBTH)

(3)



(Blue-colored complex)

(1)

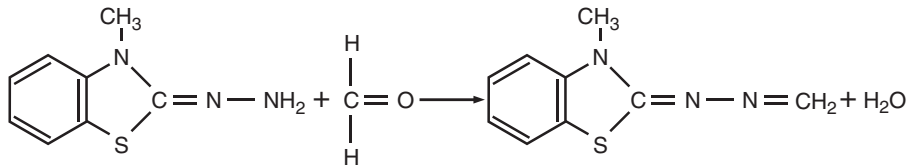


Figure 60 Chemical reaction for the MBTH method

Hardness

For water, wastewater and seawater

EDTA Titration and Calmagite Colorimetric Methods

Introduction

Hardness in water is caused by dissolved minerals, primarily divalent cations, including calcium (Ca^{2+}), iron (Fe^{2+}), strontium (Sr^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}). Calcium and magnesium ions are usually the only ions present in significant concentrations; therefore, hardness is generally considered to be a measure of the calcium and magnesium content of water. Considerations should be given when other cations contributing to hardness are present in significant amounts.

Titration methods

Hardness in water can be determined quickly by titration and the use of color indicators. By proper choice of pH, total hardness (Ca^{2+} and Mg^{2+}) or the portion contributed by calcium and magnesium individually can be measured. The traditional test for hardness involves pH adjustment to 10.1 with an ammonium buffer, addition of Eriochrome Black T indicator [1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulfonic acid] and then titration with Na_2EDTA (ethylenediaminetetraacetic acid, disodium salt) solution.

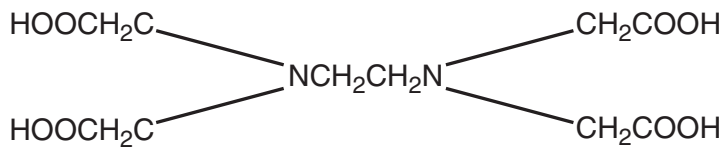


Figure 61 Chemical structure of EDTA-Ethylenediaminetetraacetic acid

Some other indicators are more stable, giving a faster reaction and a more distinct end point than Eriochrome Black T. One of the best is calmagite, 1-(1-hydroxyl-4-methyl-2-phenylazo)-2-naphthol-4 sulfonic acid, which is used in Hach total hardness tests.

Colorimetric method

The Colorimetric Method is for low level measurement of hardness. The interference of some metals with the Titration Methods will be rendered inconsequential after diluting the sample to bring it into the range of this test. Calmagite indicator and two chelating agents, EGTA and EDTA, are used in the test.

Chemical reactions

Total hardness

Several solutions including digital titrator cartridges are described in the following section for titrating prepared water samples containing calmagite indicator. TitraVer™ Hardness Titrant (0.020 N EDTA) is the most widely used. Other strengths of TitraVer Hardness Titrant are available for titrating high hardness samples. HexaVer™ Hardness Titrant also is available. HexaVer is CDTA (cyclohexanediaminetetraacetic acid, disodium salt). It gives slightly sharper end points and can tolerate higher levels of iron interference than TitraVer.

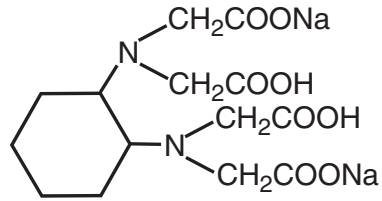
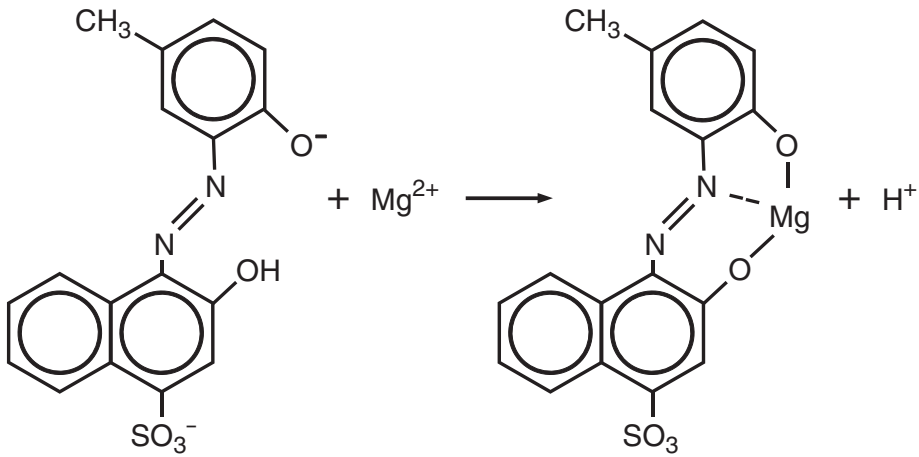


Figure 62 Chemical structure of CDTA, disodium salt

Calmagite indicator is available in special formulations as ManVer™ and UniVer™. The ManVer formulations of calmagite have been specially prepared to enhance stability and to be free from most interferences. Interferences caused by metal ions, such as copper or iron, can be removed or masked by the use of the magnesium salt of CDTA. It is effective, yet safe to use. Cyanide compounds also may be used to overcome interferences. Their use is avoided where possible because of potential environmental and health hazards.



Calmagite (blue) Calmagite-Mg complex (wine red)

Figure 63 Reaction between magnesium and calmagite indicator

The reaction of calmagite is pH-dependent; it has been determined that a pH of 10.1 is ideal. Traditionally, ammonia buffers have been used; however, they have a strong odor. Hach methods use Hardness 1 Buffer (2-amino-2-methyl-1-propanol), which is stable, safe to use and has a less objectionable odor.

The sequence of analysis in the hardness tests begins with pH adjustment and addition of inhibitors followed by formation of the Mg^{2+} and Ca^{2+} complexes with calmagite. The calcium forms a weak complex with calmagite at this pH. The solution is titrated with TitraVer (EDTA) or HexaVer (CDTA). The titrant first complexes any calcium, then magnesium. Color change from wine red to blue is an indication that all calcium and magnesium have been removed from the calmagite and complexed with the titrant.

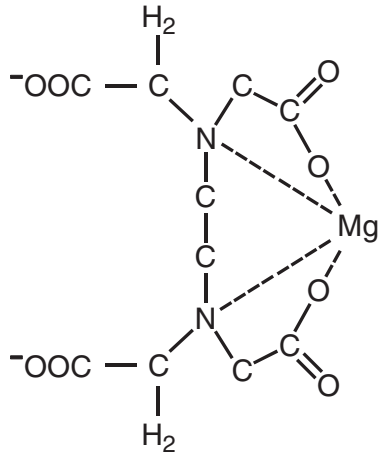


Figure 64 Magnesium complexed with TitraVer

Expression of results of the hardness titration is mg/L as $CaCO_3$. The reaction of TitraVer with Ca^{2+} and Mg^{2+} is a 1:1 ratio.

Calcium hardness

The test for calcium hardness is very similar to the total hardness test. Traditionally, either murexide indicator (ammonium purpurate) or Eriochrome Blue-Black R indicator is followed by titration with EDTA. CalVer 2 Calcium Indicator has been developed by Hach to replace these indicators. CalVer 2 (hydroxy naphthol blue) is more sensitive and has a sharper end point color change.

CalVer 2 Calcium Indicator forms a red-violet complex with calcium and changes to pure blue after TitraVer removes calcium from the complex. The pH is elevated to at least 13 to precipitate magnesium. A few drops of Magnesium Standard Solution may be added to the reaction to sharpen the end point color change. This may seem inconsistent because magnesium is precipitated by elevating the pH. However, the added magnesium is chelated preferentially by the dye and the quantity of chelated magnesium is very small; thus any error caused by addition of magnesium is negligible.

The pH adjustment is accomplished by addition of potassium hydroxide prior to addition of CalVer 2. Potassium cyanide may also be added to complex interfering metals prior to the addition of CalVer 2.

Calcium hardness and total hardness may be determined sequentially using the same sample. After the calcium hardness is determined the sample pH can be adjusted downward, using sulfuric acid. Then Hardness Buffer 1 and ManVer 2 are added and titration with TitraVer is resumed.

DO NOT use this procedure if potassium cyanide has been used in determining calcium hardness! The addition of sulfuric acid will cause deadly hydrogen cyanide gas to evolve.

Colorimetric method

Calmagite, contained in Calcium and Magnesium Indicator Solution, is added to a sample and the pH is elevated to about 12.5 by using a buffer. Adding calmagite prior to pH adjustment prevents the calcium and magnesium precipitation that ordinarily would occur at this elevated pH. The sample is then split into three equal portions.

EDTA is added to the first portion to sequester calcium and magnesium, thereby breaking the Ca- and Mg-calmagite complexes. This solution is used as a zero reference blank to standardize the spectrophotometer.

EGTA or ethyleneglycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid, is added to the second sample portion. EGTA selectively chelates calcium under conditions of the test; only absorbance due to the Mg-calmagite complex remains to be measured. The result is expressed as mg/L Mg as $CaCO_3$. After measurement, the spectrophotometer is adjusted to read "zero" on this portion.

Absorbance of the third sample portion (containing no chelant) is measured to determine mg/L Ca as $CaCO_3$. Adjust the spectrophotometer to a reading of zero after measurement of the second sample portion to compensate for absorbance due to magnesium in the sample.

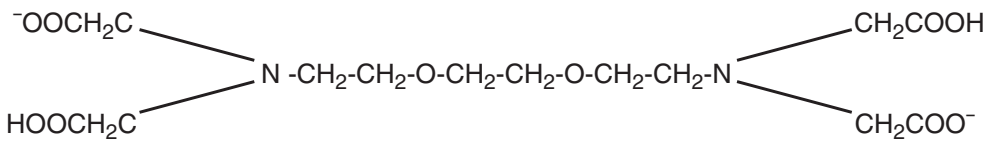


Figure 65 Chemical structure of EGTA

Hydrazine

For water and boiler water **p-Dimethylaminobenzaldehyde Method**

Introduction

Hydrazine is used as an oxygen scavenger for high pressure boilers in power plants and other industries to reduce corrosion of metal pipes and fittings. The test for hydrazine is a modification of the p-Dimethylamino-benzaldehyde Method, in which several solutions have been formulated into a single, stable reagent called HydraVer™ 2 Hydrazine Reagent. The method is both sensitive and easy to perform. It is used mostly for the determination of small amounts of hydrazine in boiler feedwater. There are no common interferences.

Chemical reactions

Under acid conditions, hydrazine combines with p-Dimethylaminobenzaldehyde to form a yellow-colored azine complex. Color development follows Beer's law and is stable after maximum color is developed in 10 to 15 minutes.

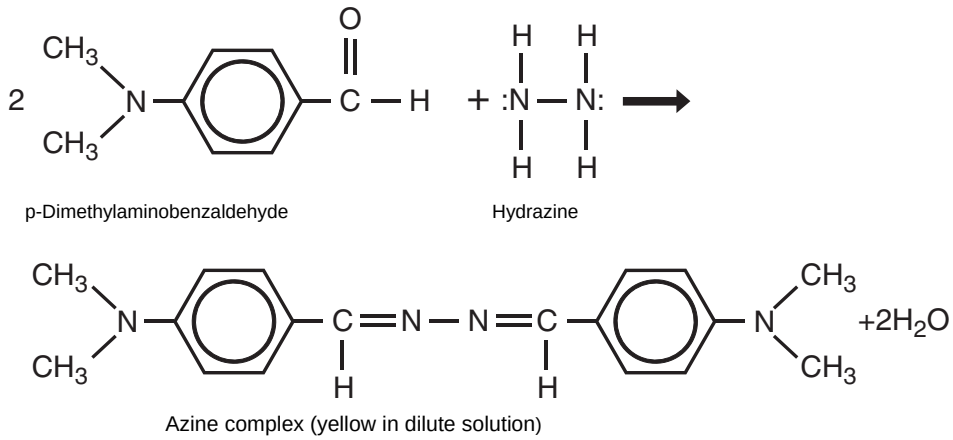


Figure 66 Chemical reaction for p-Dimethylaminobenzaldehyde method

Iron

For water and seawater

1,10-Phenanthroline, FerroZine, TPTZ and Titration Methods

Introduction

Natural waters contain variable, but minor, amounts of iron, despite its universal distribution and abundance. Iron in ground waters is normally present in the ferrous (Fe^{2+}), or soluble state, which oxidizes easily to ferric (Fe^{3+}) iron on exposure to air. Iron can enter a water system from leaching of natural deposits, iron-bearing industrial wastes, effluents of pickling operations, or from acidic mine drainage.

Iron in domestic water supply systems stains laundry and porcelain, causing more of a nuisance than a potential health hazard. Taste thresholds of iron in water, 0.1 mg/L for Fe^{2+} and 0.2 mg/L for Fe^{3+}, result in a bitter or astringent taste. Water used in industrial processes must contain less than 0.2 mg/L of total iron.

Three methods of colorimetric iron analysis are used in Hach procedures. The 1,10-Phenanthroline Method is the best-known test for iron. The Fe^{2+} procedure uses Ferrous Iron Reagent Powder containing 1,10-Phenanthroline as an indicator. Total iron determination or analysis uses FerroVer Iron Reagent. FerroVer Iron Reagent contains 1,10-Phenanthroline, combined with a reducing agent, to convert all but the most resistant forms of iron present in the sample to Fe^{2+}.

The FerroZine Method for total iron is more than twice as sensitive as the 1,10-Phenanthroline Method. Researchers at Hach have patented a process to manufacture high purity FerroZine Iron Reagent, ideal for iron measurement, in economical quantities. FerroZine is highly specific for iron, forms an intensely-colored stable complex and performs in the pH range of 3–7.5. The FerroZine Method requires boiling to dissolve rust.

The TPTZ Method for total iron has the advantages of simplicity, sensitivity and freedom from common interferences. Iron in the sample, including precipitated or suspended iron such as rust, is converted to Fe^{2+} by a reducing agent. A highly colored Fe^{2+}-TPTZ complex is formed.

Hach Methods also include a high-range titration procedure utilizing sulfosalicylic acid as the indicator and EDTA as the titrant.

Chemical reactions

1,10-Phenanthroline method

1,10-Phenanthroline, contained in Ferrous Iron Reagent Powder, reacts with Fe^{2+} to form a characteristic orange-colored complex. The intensity of color development is directly proportional to the amount of Fe^{2+} in the sample. Total iron also can be determined with FerroVer Iron Reagent. (When Environmental Protection Agency reporting is necessary, digestion of the sample is also required)

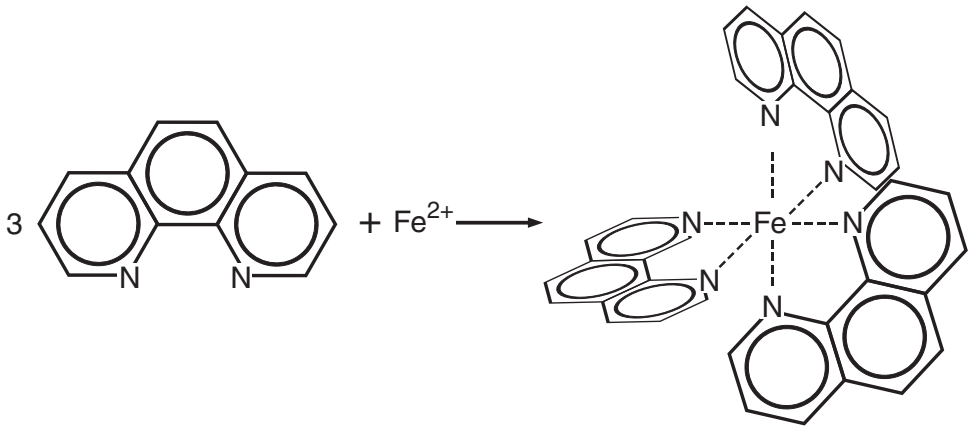


Figure 67 Chemical reaction for 1,10-Phenanthroline method

FerroZine method

Very low concentrations of iron can be determined using an ultra-sensitive iron indicator, FerroZine Iron Reagent, 3-(2-pyridyl)-5, 6-bis (4-phensylsulfonic acid)-1, 2, 4-triazine, monosodium salt. FerroZine Iron Reagent also can be used to analyze samples containing magnetite (black iron oxide) or ferrites. The test is performed by adding a solution of FerroZine Iron Reagent to the water sample. The sample is thereby buffered to a pH of 3.5 and a purple-colored complex directly proportional to the iron concentration is formed. A reducing agent is included to convert any Fe^{3+} to Fe^{2+}(which forms the colored complex).

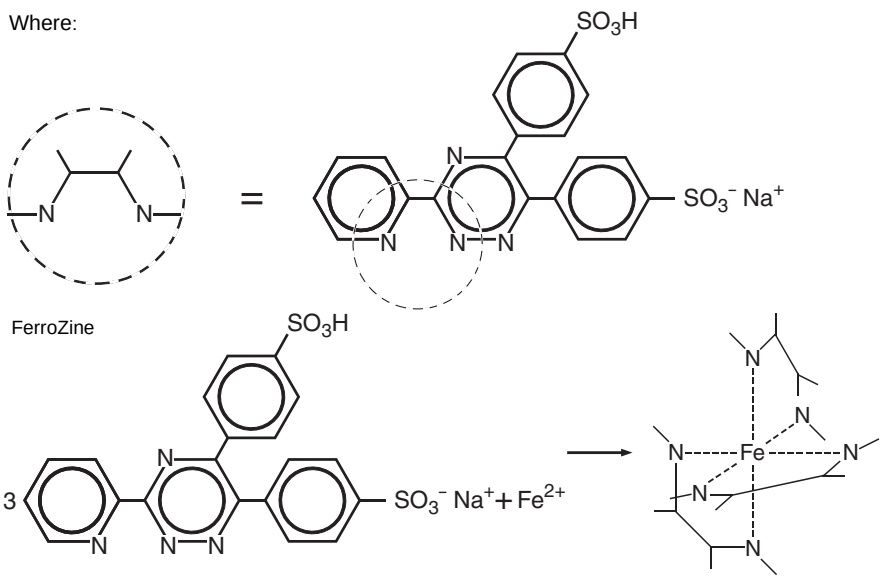


Figure 68 Chemical reaction for FerroZine method

Titration method

The Titration Method is intended for high iron concentrations, such as oil-field water determinations. In this method the iron present in the sample is oxidized to Fe^{3+} by an oxidizing agent. The Fe^{3+} is then detected with sulfosalicylic acid, which forms a wine red complex with Fe^{3+}. The solution is titrated with TitraVer (EDTA) to a colorless to yellow end point. A buffer is added to stabilize the Fe^{3+}

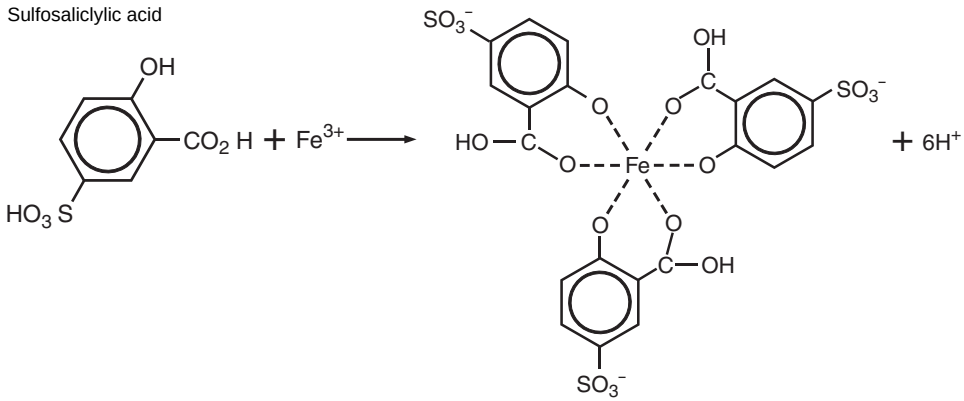


Figure 69 Titration method

TPTZ method

TPTZ, 2,4,6-tripyridyl-s-triazine, reacts with Fe^{2+} to form a deep blue-purple color. Reducing agents are added to convert iron in the sample to the Fe^{2+} form. TPTZ, reducing agents and pH buffers are combined in one simple reagent— TPTZ Iron Reagent Powder Pillows.

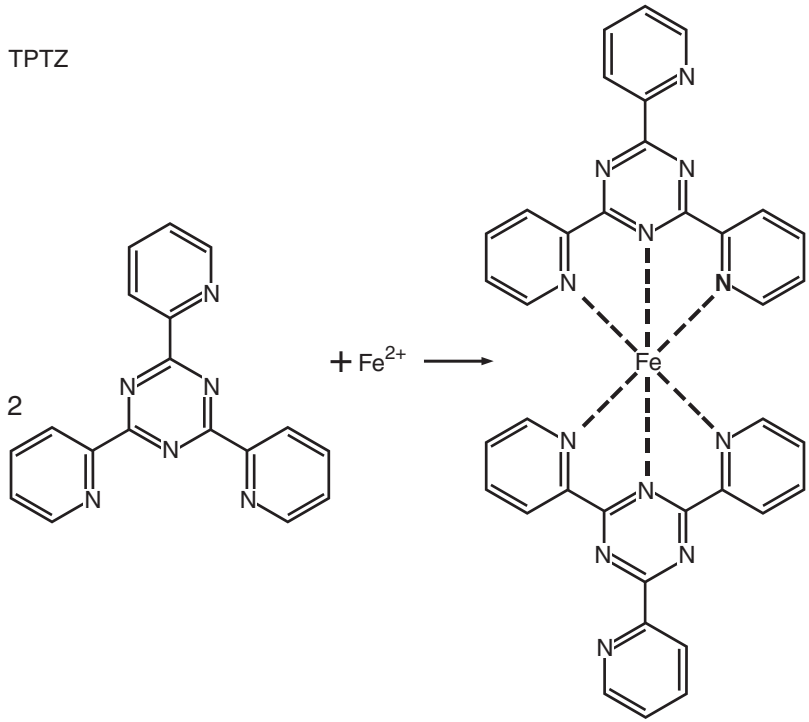


Figure 70 Chemical reaction for TPTZ method

Langelier and Aggressive Indices

Method 8073

Langelier saturation index

The Langelier Saturation Index (LI), a measure of a solution's ability to dissolve or deposit calcium carbonate, is often used as an indicator of the corrosivity of water. The index is not related directly to corrosion, but is related to the deposition of a calcium carbonate film or scale; this covering can insulate pipes, boilers and other components of a system from contact with water. When no protective scale is formed, water is considered to be aggressive and corrosion can occur. Highly corrosive water can cause system failures or result in health problems because of dissolved lead and other heavy metals. An excess of scale can also damage water systems, necessitating repair or replacement.

In developing the LI, Langelier derived an equation for the pH at which water is saturated with calcium carbonate (pH_S). This equation is based on the equilibrium expressions for calcium carbonate solubility and bicarbonate dissociation. To approximate actual conditions more closely, pH_S calculations were modified to include the effects of temperature and ionic strength.

The Langelier Index is defined as the difference between actual pH (measured) and calculated pH_S. The magnitude and sign of the LI value show water's tendency to form or dissolve scale and thus to inhibit or encourage corrosion.

Although information obtained from the LI is not quantitative, it can be useful in estimating water treatment requirements for low pressure boilers, cooling towers and water treatment plants, as well as serving as a general indicator of the corrosivity of water.

Parameter measurement

The Langelier Saturation Index can be calculated easily by using Hach products to determine the pH of calcium carbonate saturation (pH) and the actual pH of a solution. Using a simple formula, the pH is derived from the values for calcium hardness, total alkalinity at pH 4.5, temperature and total filterable residue (total dissolved solids). All of these procedures are included in this manual.

Temperature:
Temperature can be measured in degrees celsius with a laboratory thermometer (Catalog. Number. 566-01). If only a Fahrenheit thermometer is available, conversion to degrees Celsius will be necessary.
[°C = 5/9 x (°F – 32)].

Calculation
After the preceding parameters have been determined, calculate the pH from the following formula:

$$pH_S = A + B - C - D$$

Where:
Constant A takes into account the effect of temperature. It is found by selecting the value from the *Water temperature* table that corresponds to the measured temperature in degrees Celsius.

Constant B is a correction for the ionic strength of the sample. It is determined using the *TDS* table by taking the value that corresponds to the measured total filterable residue or the estimated total dissolved solids (TDS).

Value C is obtained from the *Hardness or alkalinity* table by reading the value corresponding to the calcium hardness (in mg/L $CaCO_3$) of the sample.

Value D is obtained from the *Hardness or alkalinity* table by reading the measured value for total alkalinity (in mg/L $CaCO_3$) of the sample.

The Langelier Saturation Index is the difference between the actual pH of the solution and pH_s calculated above.

$$LI = pH_{actual} - pH_S$$

Interpretation

The LI is a gauge of whether a water will precipitate or dissolve calcium carbonate. If the pH_s is equal to the actual pH, the water is considered “balanced”. This means that calcium carbonate will not be dissolved or precipitated. If the pH_s is less than the actual pH (the LI is a positive number), the water will tend to deposit calcium carbonate and is scale-forming (nonaggressive). If the pH_s is greater than the actual pH (the LI is a negative number), the water is not saturated and will dissolve calcium carbonate (aggressive). In summary:

$pH_S = pH_{actual}$, water is balanced

$pH_S < pH_{actual}$, LI = positive number, water is scale forming (nonaggressive)

$pH_S > pH_{actual}$, LI = negative number, water is not scale forming (aggressive)

It is important to remember that the LI value is not a quantitative measure of calcium carbonate saturation or corrosion.

Because the protective scale formation is dependent on pH, bicarbonate ion, calcium carbonate, dissolved solids and temperature; each may affect the water’s corrosive tendencies independently. Soft, low-alkalinity waters with either low or excessively high pH are corrosive, even though this may not be predicted by the LI. This is because insufficient amounts of calcium carbonate and alkalinity are available to form a protective scale.

Waters with high pH values and sufficient hardness and alkalinity may also be corrosive, even if the LI predicts the opposite. This is the result of calcium and magnesium complexes that cannot actively participate in the scale forming process. Analytical procedures do not distinguish between these complexes and available calcium and magnesium; therefore, the LI value is not accurate in such situations.

Corrosive tendencies may also be exhibited by water containing high concentrations of sulfate, chloride and other ions which interfere with uniform carbonate film formation.

As a result of these and other problems, the LI is useful only for determining the corrosivity of waters containing more than 40 mg/L of alkalinity, sufficient calcium ion concentration and ranging between pH 6.5 and 9.5.

Aggressive index

The Aggressive Index (AI), originally developed for monitoring water in asbestos pipe, is sometimes substituted for the Langelier Index as an indicator of the corrosivity of water. The AI is derived from the actual pH, calcium hardness and total alkalinity. (Use procedures contained in this handbook). Where it is applicable, it is simpler and more convenient than the LI. Because the AI does not include the effects of temperature or dissolved solids, it is less accurate as an analytical tool than the LI.

Calculation

After obtaining the pH, total alkalinity and calcium hardness, use the following formula to calculate the AI:

$$AI = pH_{actual} + C + D$$

Where:

Value C is obtained from the *Hardness or alkalinity* table by reading the value corresponding to the calcium hardness (in mg/L $CaCO_3$) of the sample.

Value D is obtained from the *Hardness or alkalinity* table by reading the measured value for total alkalinity (in mg/L $CaCO_3$) of the sample.

Interpretation

As with LI, the AI is not a quantitative measure of corrosion, but is a general indicator of the tendency for corrosion to occur and as such, should be used with proper reservation. An AI of 12 or above indicates nonaggressive (not corrosive) water. AI values below 10 indicate extremely aggressive (corrosive) conditions. Values of 10–11.9 suggest that the water is moderately aggressive. Corrosivity characteristics of water as indicated by the LI and AI are compared in the *Corrosion characteristics* table.

Table 430 Water temperature

Water temperature, °C	A
0	2.60
4	2.50
8	2.40
12	2.30
16	2.20
20	2.10
25	2.00
30	1.90
40	1.70
50	1.55
60	1.40
70	1.25
80	1.15

Table 431 TDS

TDS, mg/L	B
0	9.70
100	9.77
200	9.83
400	9.86
600	9.89
1000	9.90

Table 432 Hardness or alkalinity

Calcium hardness or total alkalinity in mg/L $CaCO_3$	C[1] or D[2]
10	1.00
20	1.30
30	1.48
40	1.60
50	1.70
60	1.78
70	1.84
80	1.90
100	2.00
200	2.30
300	2.48
400	2.60
500	2.70
600	2.78
700	2.84
800	2.90
900	2.95
1000	3.00

[1] Factor C is the logarithm (base 10) of the calcium hardness expressed in mg/L

[2] Factor D is the logarithm (base 10) of the total alkalinity expressed in mg/L

Table 433 Corrosion characteristics

Corrosive characteristics	Langelier index	Aggressive index
Highly aggressive	< –2.0	< 10.0
Moderately aggressive	–2.0 to 0.0	10.00 to 12.0
Nonaggressive	>0.0	>12.0

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Lead

For water and wastewater **LeadTrak™ Method**

Introduction

Lead is seldom found in ground water in more than trace quantities; it averages around 10 µg/L. Surface waters contain very low levels of lead because it is precipitated by a variety of substances. Lead may be found in potable water systems as a result of the corrosion of lead service lines, lead-based solder joints, or lead-based plumbing fixtures.

Lead and its compounds are poisonous and accumulate in the bone structure when ingested in amounts exceeding the natural elimination rate of about 300 micrograms per day. Accumulation of significant amounts of lead in the body may cause severe and permanent brain damage, convulsions and death. Environmental concern with lead poisoning has resulted in a national program to reduce the concentration of lead in consumer products.

Chemical reactions

LeadTrak method

The LeadTrak™ Method for determining soluble lead as Pb^{2+} in potable water first involves the acidification of the sample to keep all lead ions soluble and to prevent the lead from being lost by precipitation or absorption on the sample container walls. Complexing and buffering agents are then added to the sample to modify the lead ions into a form which allows them to be retained by the cellulose medium in the concentrator column. Other competing ions, such as iron, copper and zinc, pass through the column and are eliminated.

The lead ions are then eluted from the concentrator column with a nitric acid solution. The nitric acid eluant is neutralized and reacted with meso-tetra (4-N-methylpyridyl) porphine tetratosylate to form a faintly colored complex. The absorbance of the complex is measured at 477 nm. EDTA is then added to the complex. The EDTA complexes with the lead and removes the lead from the porphine complex. The absorbance of the sample is read again and the lead concentration is determined by the difference between the two readings.

Manganese

For water and wastewater

Periodate Oxidation and PAN Methods

Introduction

Manganese is present in ground waters primarily as the divalent ion (Mn^{2+}), due to the lack of subsurface oxygen. Surface waters may contain combinations of manganese in various oxidation states as soluble complexes, or as suspended particles.

The occurrence of manganese in public water supplies presents more of an economic problem than a potential health hazard. Manganese causes dark stains in laundry and on plumbing fixtures, tends to deposit in water lines, and imparts an objectionable taste to beverages such as coffee and tea. Manganese levels in natural waters rarely exceed 1 mg/L, but levels of 0.1 mg/L are sufficient to cause the taste and staining problems. The recommended allowable manganese level in public water supplies is 0.05 mg/L.

Two methods for manganese determination are used in test procedures. The Periodate Oxidation Method gives a simple, rapid test for high levels of manganese. The 1-(2-Pyridylazo)-2-Napthol (PAN) Method is a sensitive, rapid procedure for low levels of manganese.

Chemical reactions

Periodate oxidation method

Manganese is oxidized to permanganate using periodate in a slightly acidified water sample. No indicator is necessary. Intensity of the purple color of the permanganate ion is a direct indication of the amount of manganese present in the sample.

$$3H_2O + 2Mn^{2+} + 5IO_4^- \rightarrow 2MnO_4^- + 5IO_3^- + 6H^+$$

PAN method

The PAN method employs an Alkaline-Cyanide Reagent. PAN Indicator is added and forms an orange-red colored complex with the manganese ion.

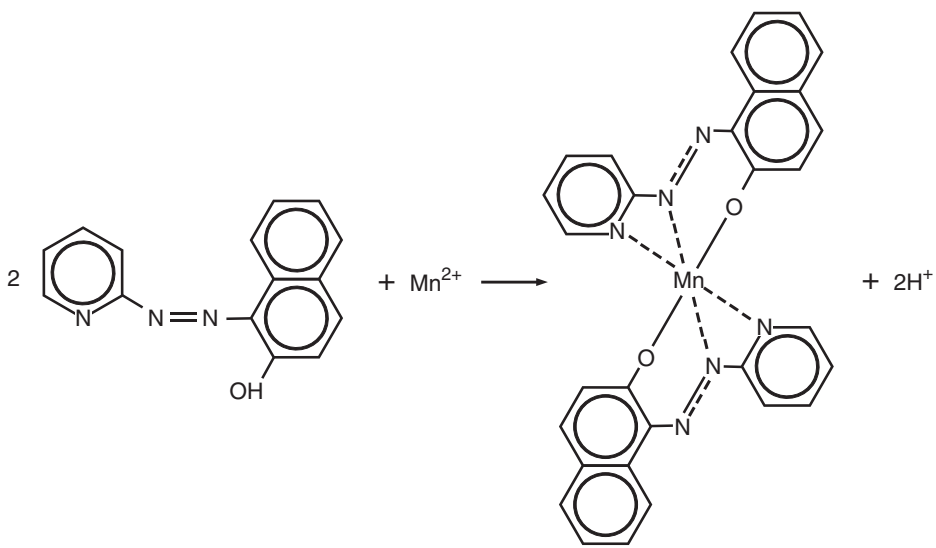

Figure 71 Chemical reaction for PAN method

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Edition 7

Mercury, Cold Vapor

Introduction

Mercury is sometimes dumped into rivers and lakes by industry. Farmers have also used mercury compounds to prevent fungi attack on seeds. In nature, bacteria change elemental mercury to organic mercury compounds that are very toxic to all living organisms. These compounds bioaccumulate in the food chain and can eventually reach humans. At high enough levels, mercury causes nerve damage, mutations and death. Because mercury is so toxic, environmental monitoring is necessary— especially at sources of industrial discharge.

Chemical reactions

The sample is digested to convert all forms of mercury to mercuric ions (Hg^{2+}) and to destroy interfering substances that may be present. The sample is then treated with a reducing agent to convert the mercuric ions to elemental mercury (Hg). The elemental mercury is volatilized from the sample in a semi-closed system by bubbling air through the sample.

The airstream containing the mercury vapor is swept through an absorber column that contains hypochlorous acid and hypochlorite ions. The vaporized mercury ions react in the column and form mercuric chloride ($HgCl_2$). The mercuric chloride is then eluted off the column with an acid solution.

The eluate is treated with HgEx™ Reagent 3 to destroy the excess hypochlorous acid and hypochlorite, and to make the solution alkaline. A sensitive indicator (HgEx 4) forms a complex with the mercuric ions, and HgEx 5 maximizes the mercury-indicator reaction, thus increasing the sensitivity of the test. The spectrophotometer is zeroed on this solution at the absorbance peak (412 nm) of the unreacted indicator. HgEx 6 is added to break the mercury-indicator bond. The analyst then measures the absorbance increase of unreacted indicator. This increase is proportional to the amount of mercury in the sample.

Molybdenum, Molybdate

For water

Mercaptoacetic Acid and Ternary Complex Methods

Introduction

Molybdenum (molybdate) salts are commonly used as corrosion inhibitors in cooling water systems. There are numerous procedures for determining molybdenum as molybdate (MoO_4^{2-}) in water. The Mercaptoacetic Acid Method is one of the most frequently referenced methods for determining molybdenum. The Hach procedure improves and simplifies this time-proven procedure.

Chemical reactions

Mercaptoacetic acid

The MolyVer™ (Mercaptoacetic Acid) Method utilizes three reagent powders. First, a MolyVer 1 Reagent Powder Pillow is added. MolyVer 1 contains a buffer to control the pH, in addition to a chelating agent to mask interferences.

Low test results can be caused from reduction of Mo^{6+} to Mo^{5+}, because the test is specific for Mo^{6+}. MolyVer 2 Reagent Powder is added to prevent reduction of the Mo^{6+} ion. Finally, mercaptoacetic acid, contained in MolyVer 3 Reagent Powder, is added. The reaction of MolyVer 3 with Mo^{6+} results in formation of a characteristic yellow color. Development of the yellow color follows Beer's Law over the range of the test.

$$MoO_4^{2-} + 2HSCH_2COOH + 2H^+ \longrightarrow$$

Molybdate — Mercaptoacetic acid

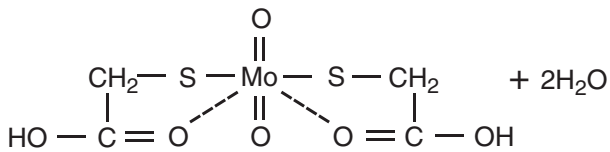


Figure 72 Chemical reaction for the Mercaptoacetic Acid method

Ternary complex method

The ternary complex method is a two-reagent method for molybdenum in the 0–3 mg/L range. First, the molybdenum-containing sample reacts with Molybdenum 1 Reagent, which contains colorimetric indicator, pH buffer, and reducing agent. The reducing agent counteracts interference by iron, a common contaminant in boiler and cooling water samples. The indicator forms a colored, binary complex with molybdenum. Depending on molybdenum levels, binary-complex color will range from pale yellow to rusty orange.

Second, Molybdenum 2 Reagent will combine with the binary complex to form an intensely colored, blue, ternary (three-part) complex directly proportional to sample molybdenum concentration. The eye perceives the color as ranging from yellow to green because the blue color is superimposed over a yellow background. These colors correspond to 0 mg/L Mo (yellow) up to 3 mg/L Mo (dark green).

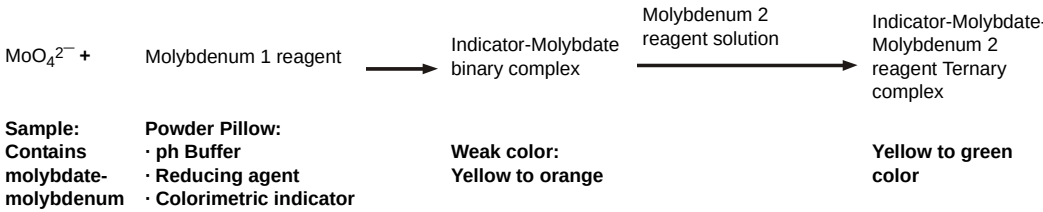


Figure 73 Chemical reaction pathway for the Ternary Complex method

Nickel

For water **Heptoxime and PAN Methods**

Introduction

Nickel is seldom found in natural waters, but often present in industrial wastewater as a direct product of metal plating baths, and as a corrosion product of stainless steel, nickel or cobalt alloys. Nickel is considered relatively nontoxic to humans. The toxicity of nickel to aquatic life varies widely and is influenced by species, pH, synergetic effects, and other factors. Nickel salts at concentrations between 0.5 and 1.0 mg/L have been shown to be toxic to some plant species.

Two methods for determining nickel, the Heptoxime Method and the PAN Method, are used in Hach procedures. The well-known heptoxime indicator has been formulated into a dry, stable powder packaged in pillows for the nickel determination. A second powder pillow is used to overcome interference from other metals present. The heptoxime forms a yellow complex, which is extracted into chloroform for measurement.

The PAN procedure is a sensitive method for detecting nickel and cobalt in concentrations less than 1 mg/L. The method is unique because simultaneous determinations of nickel and cobalt concentrations can be made on the same sample portion without the need for solvent extraction or sample preconcentration steps.

Chemical reactions

Heptoxime method

Nickel is analyzed quantitatively through its reaction with Heptoxime to form a yellow-colored complex, which is then extracted into a chloroform layer to concentrate the color and to enable a more sensitive colorimetric determination. Chelating agents are added to the sample to overcome the interferences caused by cobalt, copper and iron.

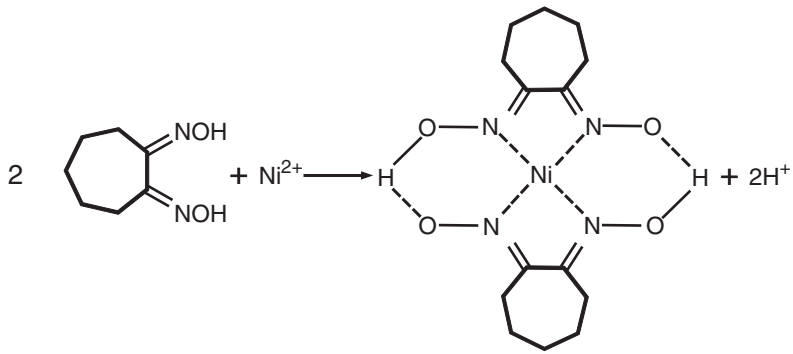


Figure 74 Chemical reactions for Heptoxime method

PAN method

The PAN method for nickel is discussed in detail in the PAN method for cobalt, because both cobalt and nickel can be determined on the same sample.

Nitrogen, Ammonia

For water, wastewater and seawater **Nessler Method and Salicylate Method**

Introduction

Ammonia is a product of the microbiological decay of animal and plant protein. It can be directly reused by plants to produce protein. Ammonia and ammonia compounds are applied directly as fertilizers.

The presence of ammonia nitrogen in surface water usually indicates domestic pollution. Ammonia in ground water is normal and is due to microbiological processes. Two methods for determining ammonia, the Nessler method and the Salicylate method, are used in Hach products and procedures.

Chemical reactions

Nessler method

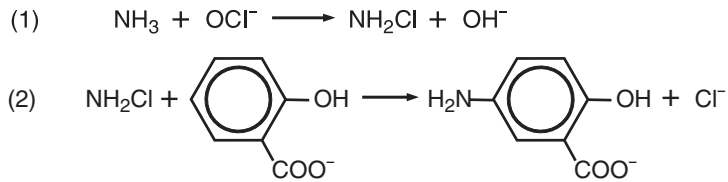
In the ammonia test, Nessler Reagent (K_2HgI_4) reacts with the ammonia present in the sample (under strongly alkaline conditions) to produce a yellow-colored species. The intensity of the color is in direct proportion to the ammonia concentration.

$$2K_2HgI_4 + NH_3 + 3KOH \rightarrow Hg_2OINH_2 + 7KI + 2H_2O$$

Salicylate method

The Salicylate method is a variation of the well-known Phenate Method, but it has an advantage of being free from mercury salts and phenol. This method is most useful for low range ammonia nitrogen determinations. Although the procedure involves multiple reactions before a final green color is developed, all reagents are contained in convenient powder pillows (Salicylate Reagent Powder Pillows and Alkaline Cyanurate Powder Pillows) or a combination of powder pillows and TNT vials.

Ammonia compounds are initially combined with hypochlorite to form monochloramine (1), which then reacts with salicylate to form 5-aminosalicylate (2).



Oxidation of 5-aminosalicylate is carried out in the presence of a catalyst, nitroprusside or $Fe(CN)_5NO^{2-}$ (also called nitroferricyanide), which results in the formation of indosalicylate, a blue-colored compound. The blue color is masked by the yellow color (from excess nitroprusside) causing a green-colored solution. The intensity of the color is directly proportional to the ammonia concentration in the sample.

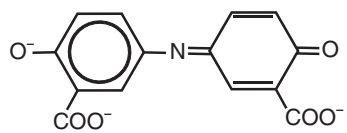


Figure 75 Chemical structure of Indosalicylate

Nitrogen, Kjeldahl

For water and wastewater **Peroxide Digestion Method**

Introduction

The test for Kjeldahl nitrogen, also referred to as crude protein, is used to determine ammonia and organic nitrogen present in a sample. Only small fractions of nitrite and nitrate nitrogen are included in the test. A preliminary digestion is used to oxidize carbon compounds to carbon dioxide, and to convert organic forms of any nitrogen present (amino acids, proteins, peptides) to ammonia. The traditional digestion uses sulfuric acid and various combinations of metallic catalysts and salts. Digestion of at least 2 hours is followed by addition of sodium hydroxide to the digest and then distillation of the ammonia into a boric acid or buffer solution. Ammonia in the distillate is measured with back titration or nesslerization. This procedure requires several hours for reagent preparation, digestion, distillation and final measurement.

The Hach Digesdahl Digestion Apparatus and the Peroxide Digestion Method allow completion of the Kjeldahl test within 15 minutes or less, depending on the nature of the sample. First, the sample is charred in concentrated sulfuric acid. Fifty percent hydrogen peroxide is fed into the reaction mixture, where it oxidizes organic carbonaceous matter and converts organic nitrogen into ammonium bisulfate. For example, the reaction with glycine, a simple amino acid is:

$$NH_2CH_2COOH + 2H_2O_2 + H_2SO_4 \rightarrow NH_4HSO_4 + CO_2 + 2H_2O$$

Glycine

Digestion apparatus

The Digesdahl digestion apparatus includes a fractionating column. Hydrogen peroxide, added slowly, trickles into the reaction mixture in the flask below. The temperature of the reaction is maintained near the boiling point of sulfuric acid (300 °C, 572 °F). Vapors from the reaction rise to the column where SO_2 and water vapors are drawn off by an aspirator. Hydrogen peroxide vapors condense in the column and return to the reaction mixture.

It should be noted that no metal catalysts or salts are used in digestion. The digest is suitable for nesslerization for final measurement without an intermediate distillation step. The digest is also suitable for mineral analysis of Ca, Mg, Mn, K, P and Zn. See *Nitrogen, Ammonia* for more information about the Nessler method.

Nitrogen, Nitrate

For water and wastewater **Cadmium Reduction Method**

Introduction

Nitrate represents the most completely oxidized state of nitrogen, and is commonly found in water. Nitrate-forming bacteria convert nitrites into nitrates under aerobic conditions; lightning converts large amounts of atmospheric nitrogen (N_2) directly to nitrates. Many granular commercial fertilizers contain nitrogen in the form of nitrates.

High levels of nitrate in water may indicate biological wastes in the final stages of stabilization, or run-off from heavily fertilized fields. Nitrate-rich effluents discharged into receiving waters can degrade water quality by encouraging excessive growth of algae. Drinking waters containing excessive amounts of nitrates can cause infant methemoglobinemia (blue babies). For this reason, a maximum concentration level in drinking water has been established by the USEPA in accordance with the Safe Drinking Water Act.

Two methods of analysis are used in the high range tests. The NitraVer™5 high range method is a modification of the Cadmium Reduction Method, using gentisic acid in place of 1-naphthylamine. All the necessary reagents have been combined into a single stable powder.

The Chromotropic Acid high range nitrate method involves the reaction of nitrate in a strong acid medium with chromotropic acid. The final reaction mixture is contained in the screw-capped Test 'N Tube™ vial.

The low range nitrate test is also a modification of the Cadmium Reduction Method, and uses a very sensitive chromotropic acid indicator. Both methods register nitrate and nitrite nitrogen.

Chemical reactions

High range—NitraVer 5

In the NitraVer 5 high range test, cadmium metal is used to reduce nitrates (NO_3^-) to nitrites (NO_2^-) (reaction 1). Next, the nitrite ions react in an acidic medium with sulfanilic acid to form an intermediate diazonium salt (reaction 2) which, when coupled with gentisic acid (reaction 3), forms an amber-colored compound. Color intensity of the compound is directly proportional to the nitrate concentration of the water sample.

(1) $NO_3^- + Cd + 2H^+ \longrightarrow NO_2^- + Cd^{2+} + H_2O$

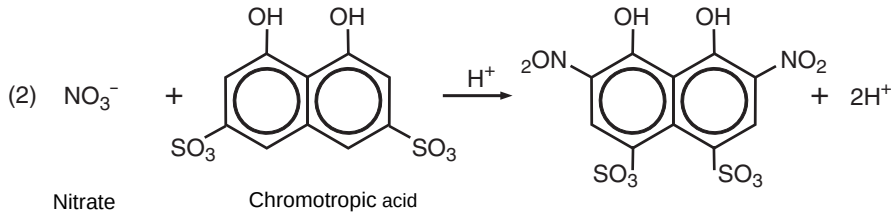
(2) $NO_2^- + H_2N\text{–}C_6H_4\text{–}SO_3H + 2H^+ \longrightarrow HO_3S\text{–}C_6H_3\text{–}N^+\equiv N + 2H_2O$

Sufanilic Acid

Diazonium Salt

High range—Chromotropic acid method

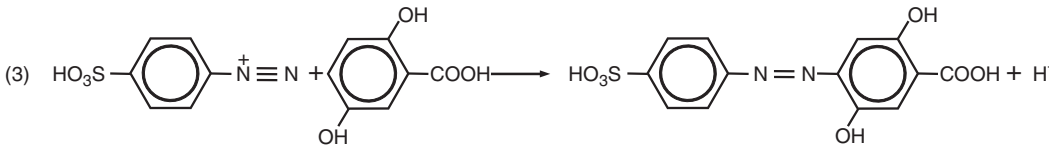
In the Chromotropic Acid test, sample is added to a Test 'N Tube™ vial containing sulfuric acid. This sample/sulfuric acid mixture is used to zero the spectrophotometer. Chromotropic acid is then added as NitraVer X Reagent B. Two moles of nitrate react with one mole of chromotropic acid to form a yellow reaction product, which exhibits maximum absorbance at 410 nm.



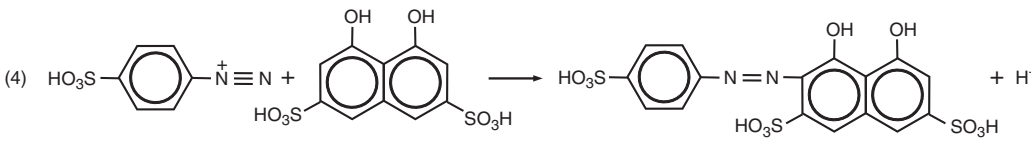
Nitrate — Chromotropic acid — Yellow color

Low range

In the low range nitrate test, cadmium metal is used to reduce the nitrates to nitrites. The cadmium is provided in NitraVer 6 Reagent Powder Pillows. Nitrite ions react with sulfanilic acid to produce an intermediate diazonium salt, as in the high range test. The diazonium salt then forms a red-orange complex with chromotropic acid. The color intensity is in direct proportion to the nitrate concentration in the sample (reaction 4). In the low range test the sulfanilic acid and chromotropic acid are contained in NitriVer 3 Reagent Powder Pillows.



Diazonium salt — Gentisic acid — Amber colored species



Diazonium Salt — Chromotropic Acid — Red-Orange color

Nitrogen, Nitrite

For water and wastewater **Ferrous Sulfate and Diazotization Methods**

Introduction

Nitrite nitrogen occurs as an intermediate stage in the biological decomposition of compounds containing organic nitrogen. Nitrite-forming bacteria convert ammonia under aerobic conditions to nitrites. The bacterial reduction of nitrates can also produce nitrites under anaerobic conditions. Nitrites are often used as corrosion inhibitors in industrial process water and cooling towers; the food industry uses nitrite compounds as preservatives.

Because nitrites readily oxidize to nitrates, they are not often found in surface waters. The presence of large quantities of nitrites indicates partially decomposed organic wastes in the water being tested. Drinking water concentrations seldom exceed 0.1 mg/L of nitrite.

The high range nitrite test is a modification of the classical brown ring test for nitrate using ferrous sulfate. By controlling the sample pH, the nitrite present is reduced to nitrous oxide, which reacts with the indicator to form a greenish-brown color. Nitrates are not registered in the test. All necessary reagents have been combined in a single powder pillow form called NitriVer 2 Nitrite Reagent Powder. A special agent helps prevent color formation or precipitation of common interfering ions.

The low range nitrite test uses chromotropic acid and sulfanilic acid as the indicator. The indicator and a buffer are combined in a single powder NitriVer 3 Nitrite Reagent. The test is sensitive to low nitrite concentrations.

Chemical reactions

High range, ferrous sulfate method

In an acidic medium ferrous sulfate reduces nitrogen in nitrite (NO_2^-) to form nitrous oxide (NO). Ferrous ions combine with the nitrous oxide to form a brown-colored complex ion, the color intensity of which is in direct proportion to the nitrite present in the water sample. Color development follows Beer's Law.

$$2Fe^{2+} + 4H^+ + 2NO_2^- \rightarrow 2Fe^{3+} + 2NO + 2H_2O$$

$$NO + FeSO_4 \rightarrow FeSO_4 \cdot NO$$

Low range, diazotization method

In the low range nitrite test, nitrite ions react with sulfanilic acid to form an intermediate diazonium salt. This reacts with chromotropic acid to produce a red-orange complex directly proportional to the amount of nitrite present. A measurement of the color intensity will provide an accurate determination of the nitrite concentration in the water sample.

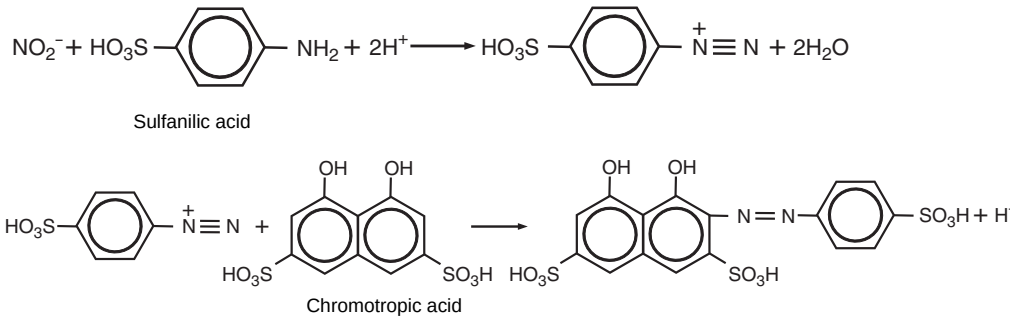


Figure 76 Chemical reaction for Low Range Diazotization method

Nitrogen, Total

For water, wastewater and seawater

Titanium Chloride and Persulfate Digestion Methods

Introduction

Total nitrogen methods measure nitrogen loads on influent streams, at intermediate stages of water treatment for sludge, and on effluent to gauge overall treatment plant efficiency. Assessing nitrogen levels allows process monitoring, adjustment and nitrogen reduction efficiency throughout the treatment.

Titanium chloride reduction method (Total Inorganic Nitrogen)
Titanium (III) ions reduce nitrate and nitrite to ammonia in a basic environment. After centrifugation to remove solids, the ammonia is combined with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate, a green solution, as in the salicylate method in Ammonia Nitrogen (see **Nitrogen, Ammonia**).

Persulfate digestion method (Total Nitrogen)
An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfate is added after the digestion to eliminate interferences from halogen oxides. Under strongly acidic conditions, nitrate reacts with chromotropic acid to nitrate the biphenyl rings at several locations, forming several nitrated products (Figure 77). The nitrated products that form are measured at 410 nm.

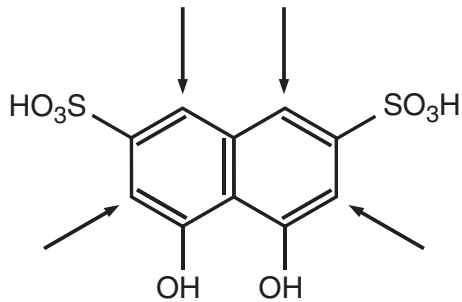


Figure 77 Chromatropic acid structure, including available reaction sites for nitrate

Organic Carbon, Total

For water and wastewater **Direct Method**

Introduction

Total Organic Carbon (TOC) testing is important in drinking water treatment as an indicator of potential disinfection by-product formation. In wastewater, TOC is valuable as a surrogate for COD testing and has applications in domestic wastewater pre-treatment standards, effluent discharge limitations, and industrial process waters.

The colorimetric TOC test measures the total amount of non-volatile organic carbon in a sample. The method is based on controlled digestion/diffusion in a sealed glass assembly*. Sample carbon is oxidized to carbon dioxide by persulfate oxidation. The carbon dioxide diffuses into a colored pH indicator solution where it is converted into carbonic acid. The resulting color change is proportional to the concentration of carbon present in the sample.

Chemical reactions

Inorganic carbon is removed from the sample by adjusting the sample to pH 2 with a buffer, and stirring vigorously for 10 minutes:

TOC = Total Carbon – Inorganic Carbon

A suitable volume of treated sample and potassium persulfate is added to a 16-mm screw top digestion vial containing Acid Digestion Solution Reagent. A 9-mm sealed glass ampule containing the TOC Indicator Solution is opened and placed inside the digestion vial. The whole assembly is then sealed with a screw cap and digested at 103–105° C (217–221 °F) for 2 hours.

In the presence of acidic persulfate and with increased pressure and elevated temperature, the sample's organic carbon is oxidized to carbon dioxide. For example, in the persulfate digestion of a sample that contains formate, the chemical reaction is:

$S_2O_8^{2-} + HCOO^- \rightarrow HSO_4^- + SO_4^{2-} + CO_2$

The evolved CO_2 then diffuses and is trapped in an aqueous solution containing a pH indicator. The absorbed CO_2 forms carbonic acid according to:

$CO_2 + H_2O \rightarrow 2H^+ + CO_3^{2-}$

The pH indicator (prior to CO_2 absorption) is in its deprotonated, or basic, form (D^-). As the absorbed CO_2 level increases, the hydrogen ion level will also increase, resulting in an increase of the protonated form of the indicator:

D^- (Color A) + $H^+ \rightarrow$ DH (Color B)

The concentration of the carbon in the sample is proportional to the color change, either the change in Color A (ΔD^-), or the change Color B (ΔDH) or the sum ($\Delta D^- + \Delta$DH).

* U.S. Patent 6,368,870

Oxygen Demand, Chemical, Mn III

For water and wastewater

Introduction

Chemical oxygen demand (COD) is defined as "a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.*" Trivalent manganese (Mn III) is a strong, non-carcinogenic chemical oxidant that changes quantitatively from purple to faint pink when it reacts with organic matter. Manganese III COD results are measured colorimetrically, and the color intensity is inversely proportional to the amount of COD in the sample.

The digestion time is 60 minutes, but can be extended when samples are difficult to completely oxidize. The reagent typically has an oxidation efficiency of about 80% for standards prepared from potassium acid phthalate and domestic wastewater samples. No oxygen demand test will oxidize all organic compounds with 100% efficiency. With non-typical samples, standards can be prepared from other reference materials. Studies have shown that the Mn III COD procedure correlates very well to biochemical oxygen demand (BOD) and dichromate COD tests.

Many COD reagents contain mercury, chromium and silver. The absence of these materials in the Mn III COD Reagent significantly minimizes the disposal cost and reduces exposure of the analyst to hazardous compounds.

Inorganic materials may interfere with the Mn III COD Reagent. Chloride is the most common interference and is removed by sample pretreatment with the Chloride Removal Cartridge. Ammonia interferes with the test when present with chloride. The interference is severe at high ammonia and chloride concentrations.

Chemical reactions

Trivalent manganese oxidizes organic materials in the sample to CO_2 and H_2O. In the process, manganese III is reduced to manganese II. The reaction occurring in the Mn III COD Reagent Vial is represented by the equation below:

$$2\ KC_8H_5O_4 + 30\ Mn_2(SO_4)_3 + 24\ H_2O \rightarrow 16\ CO_2 + 60\ MnSO_4 + 28\ H_2SO_4 + 2\ KHSO_4$$

Chemical oxygen demand results are usually expressed by the amount of oxygen consumed during the oxidation of organic matter.

When oxygen is used as the primary oxidant in the oxidation of potassium acid phthalate, the equation below describes the reaction:

$$KC_8H_5O_4 + 7.5\ O_2 \rightarrow 8\ CO_2 + 2\ H_2O + KOH$$

Seven and one-half molecules of oxygen ($O_{2)}$ consume one molecule of potassium acid phthalate (KHP). On a weight basis, the theoretical oxygen demand for KHP is 1.175 mg O_2 per mg KHP.

The interference from chloride is minimized by sample pre-treatment with the Chloride Removal Cartridge (CRC). The CRC contains a proprietary reagent to remove chloride from the sample solution. The flow rate through the CRC has been optimized for chloride removal, while at the same time minimizing any effect the CRC might have on other sample components.

* APHA Standard Methods for the Examination of Water and Wastewater, 19th ed., 1995

Suspended solids, which may contain oxidizable organic compounds, are filtered out of the sample with a glass fiber filter located in the upper part of the Chloride Removal Cartridge. After the sample has been filtered into a Mn III COD Reagent Vial, the glass fiber filter, along with any suspended solids present, is transferred into the COD Reagent Vial. The glass fiber filter is binder free and has no oxygen demand.

Comparability of the Mn III COD to other tests
For samples from a specific source, the Mn III COD results can be related empirically to BOD, dichromate COD, organic carbon, or organic matter. The test is useful for monitoring and control after correlation has been established. The test can also be used to estimate dilutions for the five day BOD test. This will ensure reliable BOD data for pre-treatment and compliance monitoring. For samples with constant chloride concentrations, reliable correlations can be developed without the chloride removal pre-treatment.

Test oxidation efficiency
Different test methods may oxidize sample components with different efficiencies. The Mn III COD Reagent will oxidize KHP standards with about 80% efficiency. Many wastewater samples are also oxidized with 80% efficiency.

For example, an ASTM Wastewater Reference Sample was analyzed for COD using both the dichromate and Mn III COD. The dichromate COD result was 1018 mg/L and Mn III COD result was 1008 mg/L. These test results are comparable despite different oxidation efficiencies because the instrument calibration is based on KHP.

Correlation of test results
To demonstrate how to correlate the results between the two different tests, a glutamic acid standard was prepared to contain a theoretical chemical oxygen demand of 500 mg/L. The dichromate COD result was 506 mg/L and the Mn III COD result was 459 mg/L. To correlate the Mn III COD results with the dichromate COD, a correlation factor is determined.

$$\frac{\text{Cr COD}}{\text{Mn III COD}} = \frac{506\ \text{mg/L}}{459\ \text{mg/L}} = 1.102\ \text{(Correlation Factor)}$$

$$\text{Mn III COD, mg/L} \times 1.102 = \text{Dichromate COD, mg/L}$$

Calibrations based on reference materials other than KHP
It may be desirable for COD results to closely match the theoretical demand of the sample. Occasionally, samples contain a major component that is incompletely oxidized. In this situation, calibration standards can be prepared from known values of that sample component. In the example above, calibration standards would be prepared from glutamic acid.

Oxygen Demand, Chemical

For wastewater

Reactor Digestion Using Potassium Dichromate Method

Introduction

The Dichromate Chemical Oxygen Demand (COD) test measures the oxygen equivalent of the amount of organic matter oxidizable by potassium dichromate in a 50% sulfuric acid solution.

Generally, a silver compound is added as a catalyst to promote the oxidation of certain classes of organics, and a mercuric compound may be added to reduce interference from the oxidation of chloride ions by the dichromate. End products are carbon dioxide, water, and various states of the chromium ion.

After the oxidation step is completed, the amount of dichromate consumed is determined titrimetrically or colorimetrically. Either the amount of reduced chromium (chromic ion), or the amount of unreacted dichromate, can be measured. If the latter method is chosen, the analyst must know the precise amount of dichromate added.

Chemical reactions

In the oxidation of organic materials by dichromate in sulfuric acid, most of the carbon is converted to carbon dioxide while any hydrogen present in the organic compound is converted to water. Other elements also may be oxidized.

Chemical oxygen demand results are usually expressed by the amount of oxygen consumed during the oxidation of organic matter. When oxygen is used as the primary oxidant in the oxidation of potassium acid pthalate, the equation below describes the reaction.

$$KC_8H_5O_4 + 7.5O_2 \rightarrow 8CO_2 + 2H_2O + KOH$$

Seven and one-half molecules of oxygen (O_2) consume one molecule of potassium acid pthalate (KHP). On a weight basis, the theoretical oxygen demand for KHP is 1.175 mg O_2 per mg KHP.

There are two basic methods, titrimetric and colorimetric, for determining the amount of chromium in a particular valence state. There are several variations of each method.

When titration is used in the measurement process, the amount of Cr^{6+} left is determined. It is done in one of two ways; in both cases, the precise initial amount of Cr^{6+} ion must be known. This is necessary because one must be able to subtract the final Cr^{6+} level from the initial level to yield the amount that was reduced to Cr^{3+}. This difference is used to calculate the COD. The initial amount is known either through calculation, because primary standard grade potassium dichromate is readily available, or by testing the bulk solution before running the individual tests.

The final amount of dichromate is most commonly determined by direct titration using ferrous ammonium sulfate as the titrant and “ferroin” (1,10-phenanthroline ferrous sulfate) as the indicator. The Fe^{2+} in the titrant reacts with the chromic ions:

$$3Fe^{2+} + Cr^{6+} \rightarrow 3Fe^{3+} + Cr^{3+}$$

1,10-phenanthroline forms an intense color with Fe^{2+} but no color with Fe^{3+}. When reduction of Cr^{6+} to Cr^{3+} is complete, Fe^{2+} reacts to form the ferroin complex and the solution color changes sharply from greenish-blue to orange-brown, signaling the end point. The end point also can be detected potentiometrically.

The colorimetric determination has several advantages over titration. Colorimetric determination is quicker and easier to run, and does not require additional reagents. In the Reactor Digestion Method, the digestion vials can be checked during the digestion process while they are hot to determine when no further oxidation is taking place, resulting in a shorter digestion time. When using the Reactor Digestion Method the spectrophotometer is set at 420 nm or 365 nm for the low ranges, and 620 nm for the high ranges. Low range measurement determines the remaining yellow Cr^{6+}. High range measurement determines the amount of green Cr^{3+} produced.

Oxygen, Dissolved

For water, wastewater, and seawater

Azide Modification of Winkler Method and Luminescence Measurement (LDO) Method

Introduction

The dissolved oxygen test is one of the most important analyses in determining the quality of natural waters. The effect of oxidation of wastes on streams, the suitability of water for fish and other organisms, and the progress of self-purification can all be measured or estimated from the dissolved oxygen content. In aerobic sewage treatment units, the minimum objectionable odor potential, maximum treatment efficiency and stabilization of wastewater are dependent on maintenance of adequate dissolved oxygen. Frequent dissolved oxygen measurement is essential for adequate process control.

Dissolved oxygen is essential for the survival of aquatic plant and animal life. Generally, 4–5 mg/L of dissolved oxygen content is a borderline concentration for an extended time period. For adequate game fish population, the dissolved oxygen content should be in the 8–15 mg/L range.

Dissolved oxygen concentration varies with water depth, sludge deposits, temperature, clarity and flow rate. Thus a single water sample is rarely representative of the over-all condition of a body of water.

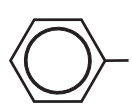
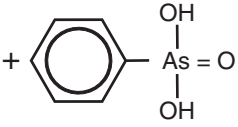
Chemical reactions

Azide modification of Winkler method

In the analysis, Mn^{2+} (manganous ion) reacts with the dissolved oxygen present in the alkaline solution to form a Mn^{4+} oxide hydroxide floc (1). Azide is added at this time to suppress interference from any nitrate present (which would react with the iodide). The solution is then acidified, and the manganese floc is reduced by iodide to produce Mn^{2+} and free iodine as I_3^-(I_2 + I^- in solution, see equation 2). The iodine gives the clear supernate a brown color. Phenylarsine oxide (PAO) or thiosulfate is then used to titrate the iodine to a colorless end point (3). (Starch indicator can be added to enhance the determination of the end point by producing a color change from dark blue to colorless.) The dissolved oxygen of the sample is then calculated from the quantity of titrant used.

(1) $2Mn^{2+} + O_2 + 4OH^- \longrightarrow 2MnO(OH)_2$

(2) $MnO(OH)_2 + 6I^- + 6H^+ \longrightarrow Mn^{2+} + 2I_3^- + 3H_2O$

(3) $2H_2O + I_3^-$  $\longrightarrow 2HI + I^-$ 

Luminescence measurement of dissolved oxygen (LDO)
The luminescence-based sensor procedure measures the light emission characteristics from a luminescence-based reaction at the sensor-water interface. A light emitting diode (LED) provides incident light required to excite the luminophore substrate. In the presence of dissolved oxygen the reaction is suppressed. The resulting dynamic lifetime of the excited luminophore is evaluated and equated to dissolved oxygen concentration.

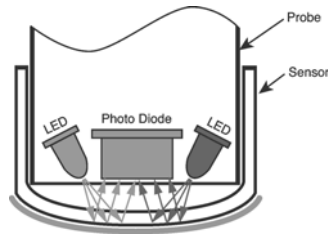


Figure 78 LDO probe

Dissolved oxygen
The *Dissolved oxygen saturation in water (mg/L)* table lists the mg/L dissolved oxygen in water at saturation for various temperatures and atmospheric pressures. The table was formulated in a laboratory using pure water. The values given are only approximations for estimating the oxygen content of a particular body of surface water

Table 434 Dissolved oxygen saturation in water (mg/L)

Temp		Pressure in millimeters and inches Hg							
		mm							
		775	760	750	725	700	675	650	625
		inches							
°F	°C	30.51	29.92	29.53	28.45	27.56	26.57	25.59	24.61
32.0	0	14.9	14.6	14.4	13.9	13.5	12.9	12.5	12.0
33.8	1	14.5	14.2	14.1	13.6	13.1	12.6	12.2	11.7
35.6	2	14.1	13.8	13.7	13.2	12.9	12.3	11.8	11.4
37.4	3	13.8	13.5	13.3	12.9	12.4	12.0	11.5	11.1
39.2	4	13.4	13.1	13.0	12.5	12.1	11.7	11.2	10.8
41.0	5	13.2	12.8	12.6	12.2	11.8	11.4	10.9	10.5
42.8	6	12.7	12.4	12.3	11.9	11.5	11.1	10.7	10.3
44.6	7	12.4	12.1	12.0	11.6	11.2	10.8	10.4	10.0
46.4	8	12.1	11.8	11.7	11.3	10.9	10.5	10.1	9.8
48.2	9	11.8	11.6	11.5	11.1	10.7	10.3	9.9	9.5
50.0	10	11.6	11.3	11.2	10.8	10.4	10.1	9.7	9.3
51.8	11	11.3	11.0	10.9	10.6	10.2	9.8	9.5	9.1
53.6	12	11.1	10.8	10.7	10.3	10.0	9.6	9.2	8.9
55.4	13	10.8	10.5	10.5	10.1	9.8	9.4	9.1	8.7
57.2	14	10.6	10.3	10.2	9.9	9.5	9.2	8.9	8.5

Table 434 Dissolved oxygen saturation in water (mg/L) (continued)

59.0	15	10.4	10.1	10.0	9.7	9.3	9.0	8.7	8.3
60.8	16	10.1	9.9	9.8	9.5	9.1	8.8	8.5	8.1
62.6	17	9.9	9.7	9.6	9.3	9.0	8.6	8.3	8.0
64.4	18	9.7	9.5	9.4	9.1	8.8	8.4	8.1	7.8
66.2	19	9.5	9.3	9.2	8.9	8.6	8.3	8.0	7.6
68.0	20	9.3	9.1	9.1	8.7	8.4	8.1	7.8	7.5
69.8	21	9.2	8.9	8.9	8.6	8.3	8.0	7.7	7.4
71.6	22	9.0	8.7	8.7	8.4	8.1	7.8	7.5	7.2
73.4	23	8.8	8.6	8.5	8.2	8.0	7.7	7.4	7.1
75.2	24	8.7	8.4	8.4	8.1	7.8	7.5	7.2	7.0
77.0	25	8.5	8.3	8.3	8.0	7.7	7.4	7.1	6.8
78.8	26	8.4	8.1	8.1	7.8	7.6	7.3	7.0	6.7
80.6	27	8.2	8.0	8.0	7.7	7.4	7.1	6.9	6.6
82.4	28	8.1	7.8	7.8	7.6	7.3	7.0	6.7	6.5
84.2	29	7.9	7.7	7.7	7.4	7.2	6.9	6.6	6.4
86.0	30	7.8	7.6	7.6	7.3	7.0	6.8	6.5	6.2
87.8	31	7.7	7.4	7.4	7.2	6.9	6.7	6.4	6.1
89.6	32	7.6	7.3	7.3	7.0	6.8	6.6	6.3	6.0
91.4	33	7.4	7.2	7.2	6.9	6.7	6.4	6.2	5.9
93.2	34	7.3	7.1	7.1	6.8	6.6	6.3	6.1	5.8
95.0	35	7.2	7.0	7.0	6.7	6.5	6.2	6.0	5.7
96.8	36	7.1	6.8	6.9	6.6	6.4	6.1	5.9	5.6
98.6	37	7.0	6.7	6.7	6.5	6.3	6.0	5.8	5.6
100.4	38	6.9	6.6	6.6	6.4	6.2	5.9	5.7	5.5
102.2	39	6.8	6.5	6.5	6.3	6.1	5.8	5.6	5.4
104.0	40	6.7	6.4	6.4	6.2	6.0	5.7	5.5	5.3
105.8	41	6.6	6.3	6.3	6.1	5.9	5.6	5.4	5.2
107.6	42	6.5	6.2	6.2	6.0	5.8	5.6	5.3	5.1
109.4	43	6.4	6.1	6.1	5.9	5.7	5.5	5.2	5.0
111.2	44	6.3	6.0	6.0	5.8	5.6	5.4	5.2	4.9
113.0	45	6.2	5.9	5.9	5.7	5.5	5.3	5.1	4.8
114.8	46	6.1	5.8	5.9	5.6	5.4	5.2	5.4	4.8
116.6	47	6.0	5.7	5.8	5.6	5.3	5.1	4.8	4.7
118.4	48	5.9	5.7	5.7	5.5	5.3	5.0	4.8	4.6
120.2	49	5.8	5.6	5.6	5.4	5.2	5.0	4.7	4.5
122.0	50	5.7	5.5	5.5	5.3	5.1	4.9	4.7	4.4

Oxygen Scavengers

For water **(DEHA) Iron Reduction Method**

Introduction

Diethylhydroxylamin (DEHA) is used as an oxygen scavenger to reduce corrosion in boilers. It is also used in photographic processes and in the manufacture of certain silicon compounds. Analytical methods to monitor DEHA concentrations have been limited to complex techniques not suitable for on-site use. Hach has developed a relatively simple test, available in both laboratory and field test kit formats.

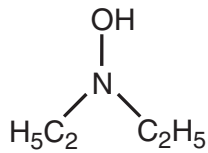


Figure 79 Chemical structure of N,N-Diethylhydroxylamine (DEHA)

Chemical reactions

DEHA reacts quantitatively with Fe^{3+} (ferric iron) and reduces it to Fe^{2+} (ferrous iron). The Fe^{2+} can then be determined by use of FerroZine™, a sensitive ferrous iron indicator.

A buffer at an optimum pH of 2.9–3.0 enhances color development. Best results are obtained if the sample temperature is 25 °C (77 °F), and with a reaction time of 10 minutes in the dark. All chemicals for this procedure are packaged in two reagents. DEHA Reagent 1 Powder Pillows combine buffer and FerroZine indicator. DEHA Reagent 2 is a liquid formulation containing a source of Fe^{3+} for the reaction. An explanation of the reaction between FerroZine and Fe^{2+} can be found in the FerroZine Method for Iron explanation.

This method is also applicable to other oxygen scavengers, such as hydroquinone, erythorbic acid (iso-ascorbic acid) methyl ethyl ketoxime and carbohydrazide, by use of the appropriate conversion factor.

Ozone

For water **Indigo Method[1]**

[1] Adapted from: Analytical Aspects of Ozone Treatment of Water and Wastewater; Lewis Publishers: Chelsea, Michigan, 1986; pages 153–156.

Introduction

Ozone (O_3), a powerful oxidant, is being increasingly used for water disinfection. It was first used in the Netherlands in the late 1800's to disinfect drinking water, and is now used worldwide in drinking water and wastewater facilities, swimming pools, spas, and in the bottled water and beverage industries. Ozone quickly provides microbial sterilization and disinfection, organic compound destruction and conversion of iron or manganese salts to insoluble oxides which can be precipitated or filtered from the water. The major reaction by-products are oxygen, water and carbon dioxide. For environmental safety, unreacted or residual ozone should be monitored.

Chemical reactions

As ozone reacts quantitatively with indigo trisulfonate (blue indigo dye), the color of the solution fades. Color intensity, inversely proportional to the amount of ozone present, is then measured at 600 nm with a photometer (colorimeter or spectrophotometer). The reagent is formulated to prevent interference from any chlorine residual which may be present.

Traditionally, ozone loss during sampling is a major cause of analytical error. Ozone is liberated when the sample is transferred from container to container, the loss causing erroneously low determinations. The evacuated AccuVac™ Ampuls draw the sample directly from the water stream or source in seconds. Ozone liberated while rushing into the Ampul is trapped there and reacts immediately with the indigo reagents. The reagent buffers the sample solution to pH 2.5. The Ampul is then placed directly in a photometer and measurements are taken in the reaction vial, eliminating cross contamination between samples.

AccuVac Ampuls for analysis cover three ranges: low range (0–0.25 mg/L), medium range (0–0.75 mg/L), and high range (0–1.50 mg/L). The lower ranges are necessary because small amounts of ozone will bleach the indigo dye only slightly. This slight decrease in color is difficult to detect if the original blue color is very intense, as it is with the high-range Ampuls.

The low and medium-range tests are designed with less intense color so that a slight bleaching can be more easily detected, thereby producing results accurate to as little as 0.01 mg/L. Conceptually the reaction may be described as:

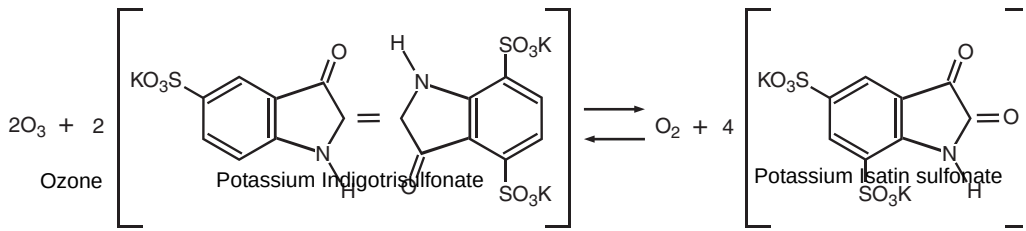


Figure 80 Chemical reaction for ozone determination, indigo method

pH

Introduction

pH is a measure of the hydrogen ion activity in a solution and is defined as: $-\log_{10} a^{H+}$ where a^{H+} is the activity of the hydrogen ion. Practically, this means that at pH 0, the hydrogen ion concentration is 1×10^{14} times greater than at pH 14. This also means the hydroxyl ion concentration at pH 14 is 1×10^{14} times greater than at pH 0. When the hydrogen and hydroxyl ions are present in equal numbers (the neutral point), the pH is 7. Values from pH 0 to pH 7 are termed Acidic and those from pH 7 to pH 14 are termed Basic. It is important to note that a pH change of one unit (for instance, from pH 6 to pH 7) actually is a factor-of-10 change (decade difference) in the hydrogen ion concentration.

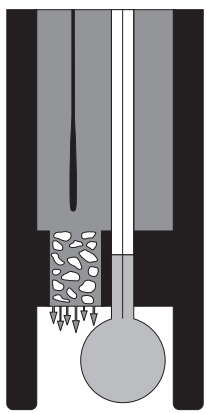
The pH electrode used in pH measurement consists of a glass sensing half-cell and a reference half-cell. Together the two half-cells form an electrode system. The sensing half-cell is a thin pH-sensitive glass membrane separating two solutions. The outer solution is the sample to be tested. The internal solution is enclosed within the glass membrane and has a known pH. An electrical potential is developed on the inside surface of the glass membrane with the internal solution and remains constant. Another electrical potential is developed on the outside surface of the glass membrane with the sample solution. This potential varies with the pH of the sample solution. The amount of variation is linear when the temperature is constant. The change in potential per pH unit is termed the Electrode Slope.

A second half-cell, or reference half-cell, in contact with the sample solution has a constant potential. Therefore, any changes in the potential of the electrode system at a given temperature will be due to changes in the pH of the sample solution. Sensing and reference half-cells may be contained in two separate electrodes, or they may be combined and called a combination electrode; see the *Platinum series combination electrode* figure.

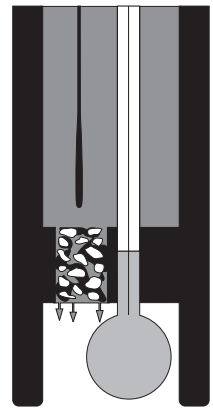
Temperature effects on pH measurements depend on the reference electrode used, pH of the solution within the pH electrode and pH of the test solution. At a certain pH, temperature will have no effect on the potential of the electrode system. This is known as the isopotential point. Also, at some pH level, the system will exhibit 0 millivolts. This is known as the zero potential point. Both the isopotential and zero potential points are features designed into electrodes. Hach electrodes are designed so the isopotential and zero potential points are at pH 7. This minimizes temperature effects in the majority of typical samples.

Conventional electrode design

The porous junction of a conventional reference half-cell is made of ceramic or fiber. With time, the junction will become clogged with silver chloride or contaminants, causing large variation in the reference potential. In addition, reference solution can be contaminated or diluted by back diffusion of sample into the junction. Particular contaminants may be introduced into the junction. Clogged or fouled junctions can cause drift along with inaccurate, noisy, erratic and sluggish pH measurements. The performance of conventional porous junctions deteriorates as they age because of clogging; see Figure 81.



New
When new, conventional porous reference junction allows electrolyte solution to flow freely.



Used
Even after just a few days, conventional reference junctions can become clogged, causing slow, unstable response and inaccurate results.

Figure 81 Conventional combination electrode

Platinum series electrode design

Platinum Series Electrodes solve this clogging problem because they use a continually renewed liquid junction, also known as a free diffusion junction (FDJ); see Figure 82. There is no ceramic or fiber plug to become clogged and therefore the electrode lasts longer. The free diffusion junction has been shown to have a faster and more stable response than conventional electrodes (Figure 81). Figure 84 shows the accuracy and stability of the Platinum Series Electrode.

Figure 85 shows the variation of the Platinum Series Electrode and a conventional electrode. The Platinum Series Electrode clearly provides repeatable results.

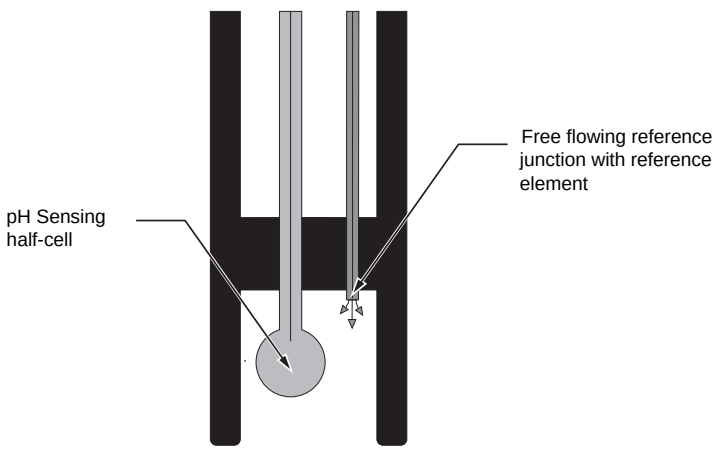


Figure 82 Platinum series combination electrode

Note: *The free-flowing reference junction design prevents clogging. Every measurement is rapid, accurate and stable, regardless of electrode age.*

The Platinum Series Electrode provides stable results in one minute in this deionized water sample. The conventional ceramic frit junction electrode shows a slow, noisy response.

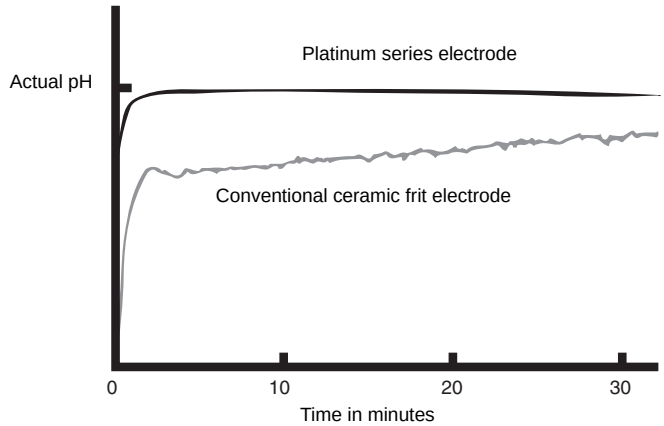


Figure 83 Response times and electrode drift for Platinum Series and conventional electrodes

The initial pH reading obtained with a conventional reference junction for a deionized water sample was incorrect, and shifted by 0.36 pH units when the sample ionic strength was increased by adding 50 mg of ultrapure KCl. The same electrode with a Platinum Series reference electrode showed significantly improved stability and accuracy.

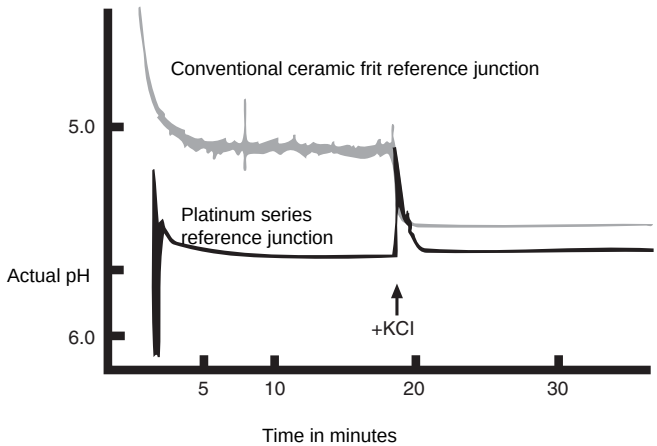


Figure 84 Accuracy of Platinum Series and conventional electrodes

Platinum series pH systems (white) provide repeatable results every time. The conventional electrode (gray) varies greatly from one measurement to the next.

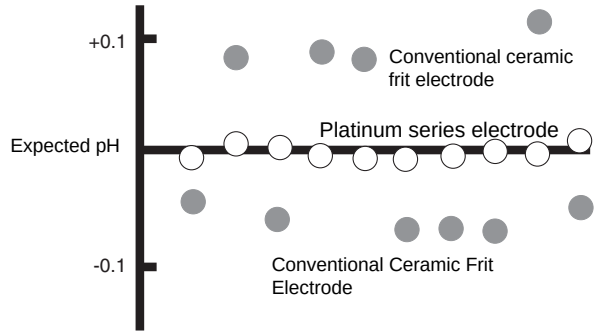


Figure 85 Repeatability comparison between Platinum Series and conventional electrodes

pH Indicators

For water and wastewater

Introduction

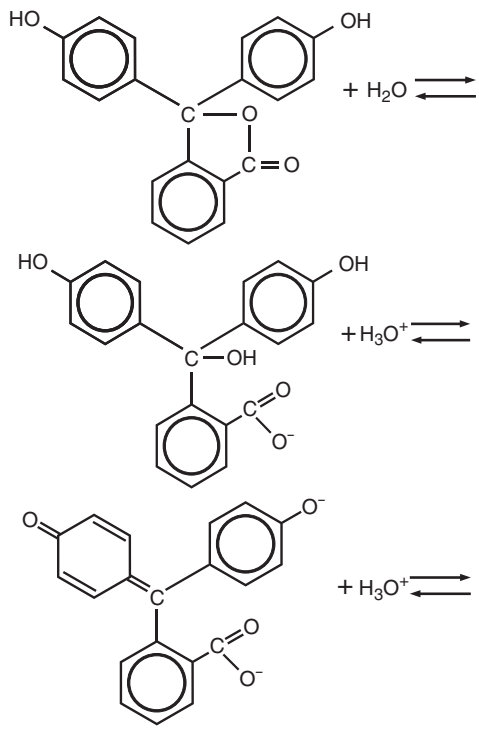
Acid-base indicators behave as weak acids or bases in water or solvents. An equilibrium established between the hydronium ion $(H_3O)^+$ and the indicator ion (In^-) can be represented as follows for an aqueous medium:

$H_2O + HIn \rightarrow H_3O^+ + In^-$ (In as a weak acid)
(acid color) (base color)

$In^- + H_2O \rightarrow InH^+ + OH^-$ (In as a weak base)
(base color) (acid color)

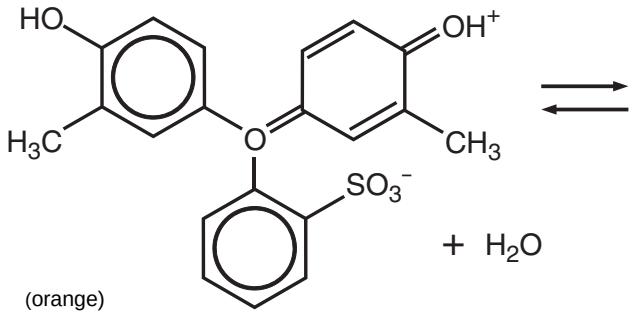
The ratio of HIn to In^- or In to InH^+ varies as a function of pH. However, the human eye cannot detect all the color changes as result of the varying concentrations of In in the solution. The color change between the predominately acid form of the indicator and the predominately base form is clearly visible, and therefore suitable for visual acid-base titration.

Most of the Hach Acid-Base Indicators can be divided into three classes based on the structure. The phthalein indicators are sparingly soluble in water but are quite soluble in alcohols. Alcohol is the preferred solvent for indicator solution preparation. Equilibrium of the phthalein-type indicators is exemplified by phenolphthalein represented as follows:

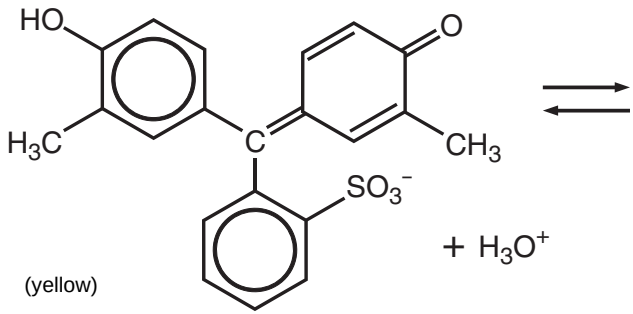


Many of the sulfonphthalein indicators exhibit two useful visual transition ranges. These indicators exhibit good stability toward strong alkali solution. Indicator solutions are usually prepared in 20% alcohol solution. The equilibria of this type of indicator are exemplified by cresol red, which is represented as follows:

Acidic transition range

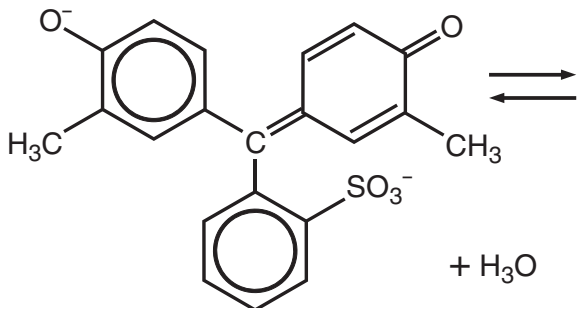


(orange)

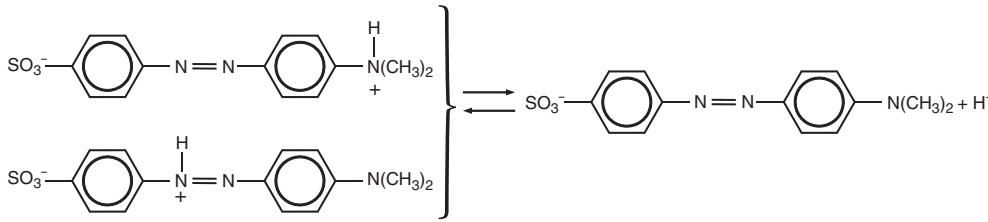


(yellow)

Basic transition range



Most of the azo indicators exhibit a color change from red to yellow. The equilibrium for azo-type indicators is exemplified by methyl orange, as shown below:



Indicator choice

The visual transition range is the main factor in selection of pH indicator. Indicator solubility also is important. When using the indicator for an acid-base titration, ease of identifying the color change and slope of the titration curve must be considered.

Figure 86 illustrates titration of a strong acid with a strong base. Any of the three indicators would be used with satisfactory results in the appropriate pH ranges. However, as the slope of the curve decreases, the selection of an indicator with the proper transition range is more important.

In Figure 87, titration of a weak acid with a strong base; only phenolphthalein would be satisfactory. Use of methyl red would give an incorrect result because the transition range of the indicator does not correspond with the actual titration equivalence point.

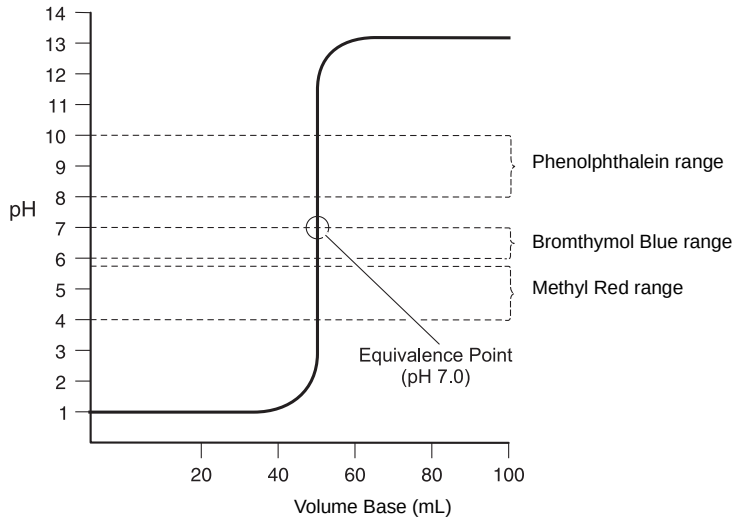


Figure 86 Titration of a strong acid with a strong base

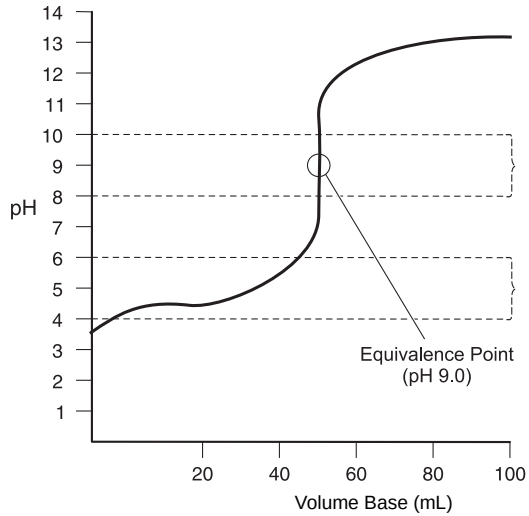
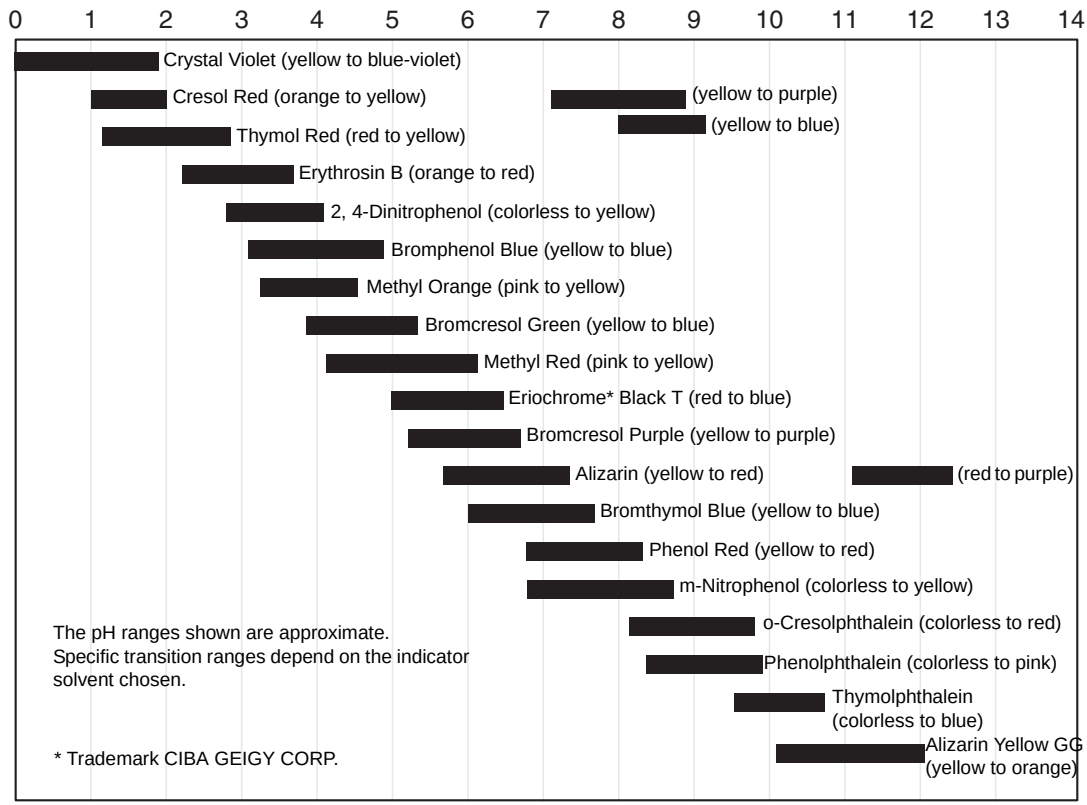


Figure 87 Titration of weak acid with a strong base

The chart below shows the ranges of some pH indicators available from Hach Company.



Phenols

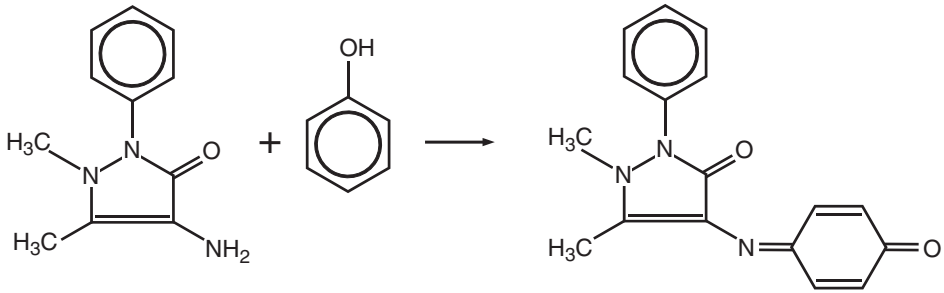
For water, wastewater and seawater **4-Aminoantipyrine Method**

Introduction

Phenols are produced as waste in oil refineries, coke plants, and in some chemical manufacturing plants. Natural waters normally contain less than 1 µg/L, but concentrations up to 20 µg/L occur in some areas. Levels of 10 to 100 µg/L phenol are detectable by taste and odor. A 1-µg/L phenol concentration can impart an objectionable taste to water following slight chlorination.

Chemical reaction

Phenols and all substituted phenols (except those with para substitution), are determined by buffering the sample to a pH of 10.0 and adding 4-aminoantipyrine to produce a yellow or amber-colored complex in the presence of ferricyanide ion. The color is intensified through extraction of the complex into chloroform. Measurement of this color quantitatively determines the phenol concentration of the sample.



4-Aminoantipyrine Phenol

Figure 88 Chemical reaction for 4-Aminoantipyrine method

Phosphonates

For water **Ultraviolet Photochemical Oxidation Method**

Introduction

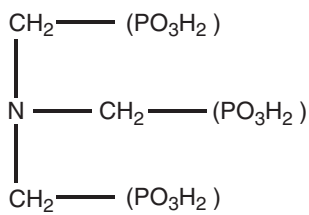
Phosphonates are employed as chemical additives to function as threshold antiscalants, corrosion inhibitors, chelants, sludge conditioners, deflocculants, dispersants and crystal growth modifiers in various industrial water treatment processes. They are used predominantly as scale and corrosion preventatives for boiler and cooling tower waters. Phosphonates exist in various formulations as acids or salts and are marketed in the form of concentrated solutions.

Until recently, analytical methods for phosphonates have been difficult, time consuming and subject to many interferences. The Ultraviolet (UV) Photochemical Oxidation Method involves a photochemical oxidation of phosphonate followed by conventional colorimetric determination of the liberated orthophosphate by the Ascorbic Acid Method. The UV Photochemical Oxidation Method is rapid, easy to use, relatively free from interferences, and applicable to both field and laboratory situations.

Chemical reactions

Phosphonic acids are organic compounds of the form $R\text{-}PO_3H_2$. Structures of two commonly used treatment chemicals are shown below; the phosphonic acid group is shown in parentheses. Phosphonates are the corresponding anions formed by ionization of one or more of the acidic hydrogens.

Aminotri
(methylene phosphonic acid)



Hydroxyethylene diphosphonic
acid

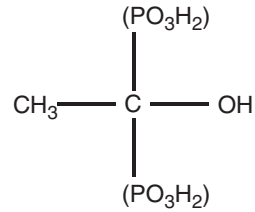
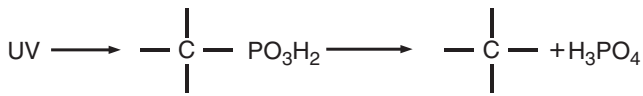


Figure 89 Chemical structures of two common phosphonic acids

Decomposition of these compounds by oxidation will liberate the organically bound phosphate as orthophosphate. The combined action of the UV radiation and oxygen will liberate orthophosphate rapidly without the necessity of heat or corrosive agents. When the photo-oxidation is carried out in the absence of acid, no significant degree of depolymerization or hydrolysis of condensed (pyro, meta or other poly) phosphates occurs, making the method a true test for organic phosphate. Presence of excess oxygen is ensured by the addition of a small amount of potassium persulfate. In this oxygen-rich environment UV light will rapidly catalyze the oxidation of the phosphonate C-P bond.



The orthophosphate formed can then be determined colorimetrically using the Ascorbic Acid method. Reagents for the Ascorbic Acid Method for orthophosphate have been combined into a single reagent powder, PhosVer™3. Determination of orthophosphate using PhosVer 3 is described in the Phosphorus methods.

Phosphorous

For water, wastewater and seawater

Amino Acid, Ascorbic Acid and Molybdovanadate Methods

Introduction

Phosphorus occurs in natural water and wastewaters almost solely as phosphates. Phosphates may enter water from agricultural run-off and as biological and industrial wastes. They may be added to water in municipal and industrial water treatment processes to control corrosion. A certain amount of phosphate is essential for most plants and animals, but too much phosphate in water can contribute to eutrophication, especially when large amounts of nitrogen are also present.

Phosphorus can be classified as orthophosphate, condensed phosphate or organically bound phosphate. Condensed phosphates are formed by dehydrating the orthophosphate radical; they include metaphosphate, pyrophosphate and polyphosphate. The only form of phosphate determined directly is orthophosphate; other forms require pretreatment for conversion to orthophosphate for analysis. When no pretreatment is used, phosphate analyses determine Reactive Phosphorus. Reactive phosphorus is a measure of orthophosphate, plus a small fraction of condensed phosphate that may have been hydrolyzed during the test.

Hach offers high and low range tests for reactive phosphorus. High range tests can be completed with the Amino Acid Method or the Molybdovanadate Method. The Molybdovanadate Method uses a single reagent and has a faster reaction than the Amino Acid Method. Both methods have a broad range and are free from most interferences. Low range tests use the Ascorbic Acid Method.

Condensed phosphates plus orthophosphate can be determined by acid hydrolysis using sulfuric acid, followed by the reactive phosphorus test for the appropriate range. A small amount of organically bound phosphorus will be included in this measurement. The results of the test are reported as acid-hydrolyzable phosphorus. Total phosphorus (orthophosphate, condensed and organically bound) can be determined by acid oxidation with persulfate, followed by the reactive phosphorus test. Organically bound phosphate can then be determined by subtracting the acid-hydrolyzable phosphorus.

Chemical reactions

Pretreatment steps

Reactions for pretreatment to determine acid-hydrolyzable and total phosphorus are illustrated below:

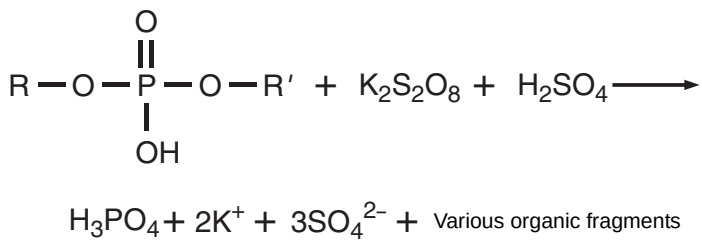


Figure 90 Example of potassium persulfate oxidation of organically bound phosphorus[1]

[1] R and R' represent various organic groups

Amino acid and ascorbic acid methods

Reactive phosphorus is determined in essentially two steps for either the Ascorbic Acid Method (low range) or the Amino Acid Method (high range). The first step involves reaction of orthophosphate with molybdate in acid solution, which forms a yellow-colored phosphomolybdate complex:

$$12MoO_3 + H_2PO_4^- \rightarrow (H_2PMo_{12}O_{40})^-$$

The phosphomolybdate complex is then reduced by either an amino acid or ascorbic acid, causing a characteristic molybdenum blue species. Various structures for the molybdenum blue species have been suggested in the literature. For example, see Killeffer, D. H., *Molybdenum Compounds-Their Chemistry and Technology*, Interscience Publishers, 1952.

All reagents for the Ascorbic Acid Method are contained in PhosVer™3 Reagent Powder Pillows. Reagents for the Amino Acid Method are contained in Amino Acid Reagent Solution and Molybdate Reagent Solution.

Molybdovanadate method

Reactive phosphorus combines with molybdate in an acid medium to form a phosphomolybdate complex. Vanadium, contained in Molybdovanadate Reagent, reacts with the complex to form vanadomolybdophosphoric acid. Intensity of the resulting yellow color is proportional to the concentration of reactive phosphorus. One possible formula for the complex is suggested below. The exact structure is not known.

$$[PO_4 \bullet VO_3 \bullet 16MoO_3]^{4-}$$

Potassium

For water and wastewater **Tetraphenylborate Method**

Introduction

Potassium, one of the most abundant elements, is found in many minerals. Soils contain approximately 1 to 4% potassium. Concentrations of potassium in most drinking water is usually less than 20 mg/L; occasionally brines may contain more than 100 mg/L. The greatest areas of interest in measurement of potassium levels probably are medicine and agriculture, due to the importance of potassium as a mineral for plants and animals. Potassium salts, particularly potash, are common in fertilizers.

The Tetraphenylborate Method for determination of potassium in water is accurate, rapid, and inexpensive. In the reaction, a precipitate is formed and the resulting increase in turbidity is measured. All necessary reagents are packaged in three powder pillows to provide reagent stability, convenience and accuracy.

Chemical reactions

Potassium combines with sodium tetraphenylborate to form potassium tetraphenylborate, a white precipitate. The precipitate remains in suspension in samples with low concentrations of potassium, causing an increase in turbidity.

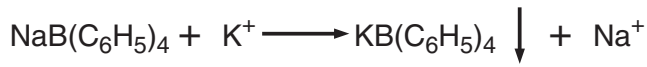


Figure 91 Chemical reaction between potassium and tetraphenylborate

The sodium tetraphenylborate is contained in Potassium 3 Reagent Powder Pillows. Ammonium salts, magnesium and calcium interfere with the precipitation. Potassium 1 Reagent Powder Pillows and Potassium 2 Reagent Powder Pillows prevent these interferences.

Selenium

For water and wastewater **Diaminobenzidine Method**

Introduction

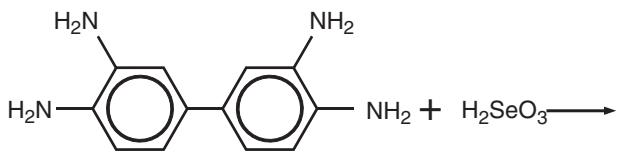
Selenium levels in natural waters seldom exceed 0.01 mg/L and concentrations greater than 0.50 mg/L are rare. The appearance of selenium in natural waters may be due to seepage from soils containing selenium or intrusion of industrial wastes.

Selenium, very toxic to man and animals, exhibits properties similar to those of arsenic. Selenium is suspected of causing dental caries and of being carcinogenic, but trace amounts also have been found essential to maintain normal body metabolism. For this reason, a maximum concentration level in drinking water has been established by the USEPA in accordance with the Safe Drinking Water Act.

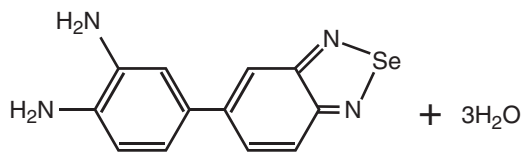
The Diaminobenzidine Extraction Method uses diaminobenzidine indicator, which develops a yellow color with selenium. This method measures only the tetravalent selenium (Se^{4+}). Selenium present as Se^{2+} and Se^{6+} is not detected unless the sample is first distilled.

Chemical reactions

In the test for selenium, an EDTA masking agent is first added to the sample to remove interferences such as iron. The addition of formic acid adjusts the sample to an optimum pH range of 1–2. Under these conditions, diaminobenzidine reacts with all selenium present in the Se^{4+} form to give a yellow-colored piazselenol complex. (Selenium existing in the selenate form is not determined by this method.) The sample is then buffered to a pH of 10–11, where the piazselenol complex can be extracted into toluene. Measurement of the color intensity directly indicates the amount of Se^{4+} in the sample.



Diaminobenzidine



Silica

For water and seawater **Silicomolybdate/Heteropoly Blue Method**

Introduction

Silicon is the second most abundant element in nature. Accordingly, it is not surprising that most waters contain compounds of silicon, usually as silica (SiO_2) or silicates (SiO^{4-} and SiO_3^{2-}). Silica concentration in water is commonly less than 30 mg/L, although concentrations greater than 100 mg/L are not unusual; concentrations exceeding 1000 mg/L are possible in brines and brackish water.

Silica and silicates are added to water for a number of uses, such as water conditioners, detergents, and corrosion inhibitors. However, silica in water can cause significant problems for industries, primarily in boiler and turbine applications. High pressures and high temperatures cause silica deposits on tubes of boilers and heat exchangers. These glassy deposits lower the efficiency of heat transfer and lead to premature failure. Silica deposits on steam turbine blades decrease efficiency and necessitate costly downtime for cleaning. Silica levels must be kept below 0.005 mg/L in very high pressure applications.

Measuring silica in water is useful when efficiency of demineralizers is monitored. Testing for silica (one of the first impurities detected when the exchange capacity of a demineralizer is exhausted) provides a sensitive check of demineralizer performance.

Analytical procedures for silica include the Silicomolybdate Method for high range measurement and the Heteropoly Blue Method for low range measurement. The Heteropoly Blue method is an extension of the Silicomolybdate method to increase sensitivity.

Chemical reactions

High and low ranges

The Silicomolybdate Method involves the reaction of molybdate ion with silica and phosphate under acid conditions to form a yellow color. Citric acid is added to destroy the phosphomolybdic acid complex (the yellow color formed due to phosphate), but not the silicomolybdic acid complex. For large amounts of silica, the remaining yellow color is intense enough to be read directly. For low concentrations, an amino-naphthol sulfonic acid reducing agent is used to convert the faint yellow color to a dark heteropoly blue species. The color formed is directly proportional to the amount of silica present in the original sample; a colorimetric measurement of this intensity provides an accurate means of determining the silica concentration. Some forms of silica (usually polymeric) will not react with ammonium molybdate and must be digested with sodium bicarbonate to be converted to a reactive form.

Silicic acid reacts with water and hydrates as follows:

$$H_2SiO_3 + 3H_2O \rightarrow H_8SiO_6$$

This hydrated silicic acid reacts with molybdate in the presence of acids to form silicomolybdic acid.

$$H_8SiO_6 + 12(NH_4)_2MoO_4 + 12H_2SO_4 \rightarrow H_8[Si(Mo_2O_7)_6] + 12(NH_4)_2SO_4 + 12H_2O$$

This silicomolybdic acid is then reduced to a blue color (heteropoly species) by an amino naphthol sulfonic acid for low concentrations.

Sulfate

For water, seawater and oil-field water **Turbidimetric Method**

Introduction

Sulfate occurs in natural waters in a wide range of concentrations. Mine waters and industrial effluents frequently contain large amounts of sulfate from pyrite oxidation and the use of sulfuric acid.

Because of sulfate's cathartic action, a secondary maximum contaminant level has been established by the USEPA, in accordance with the Safe Drinking Water Act. The taste threshold of magnesium sulfate is 400 to 600 mg/L; calcium sulfate is 250 to 800 mg/L. Sulfate may be either beneficial or detrimental in water used for manufacturing and domestic supply. The presence of sulfate is advantageous in producing desired flavors in the brewing industry. In domestic water systems, sulfates do not appear to cause any increased corrosion on brass fittings, but concentrations above 200 mg/L do increase the amount of lead dissolved from lead pipes.

Sulfate determination is important in oil field applications where two or more waters are mixed. High concentrations of sulfate, along with barium, calcium, and strontium, can form insoluble scales.

The procedure for determining sulfate is a modification of the Barium Sulfate Turbidimetric Method. A single dry powder reagent, SulfaVer™4 Sulfate Reagent, will cause a milky precipitate to form if sulfate is present. The amount of turbidity formed is proportional to the amount of sulfate present.

Chemical reactions

Sulfate is determined by its quantitative precipitation with barium chloride. Because the finely divided barium sulfate turbidity formed is proportional to the amount of sulfate in the sample, a photometric reading enables the sulfate concentration to be accurately determined.

$$Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4^{-}$$

Sulfide

For water, wastewater and seawater **Methylene Blue Method**

Introduction

Sulfide is a poisonous by-product of the anaerobic decomposition of organic matter and commonly is found in sewage and industrial wastewaters. Sulfide can be present as the free sulfide ion (S^{2-}) or as dissolved hydrogen sulfide (H_2S and HS^-). The toxicity of hydrogen sulfide is equivalent to that of hydrogen cyanide, but its offensive odor is detectable long before toxic levels are reached. However, at high concentrations hydrogen sulfide quickly deadens the sense of smell; thus toxic levels may be present but undetected.

Chemical reactions

The sulfide test is based on the ability of hydrogen sulfide and acid-soluble metallic sulfides to convert N,N-dimethyl-p-phenylenediamine directly to methylene blue in the presence of a mild oxidizing agent (potassium dichromate). Intensity of the methylene blue color development is directly proportional to the amount of sulfide present in the original sample. A colorimetric measurement of this intensity provides an accurate means to determine the sulfide concentration. All necessary reagents are contained in Sulfide 1 Reagent and Sulfide 2 Reagent.

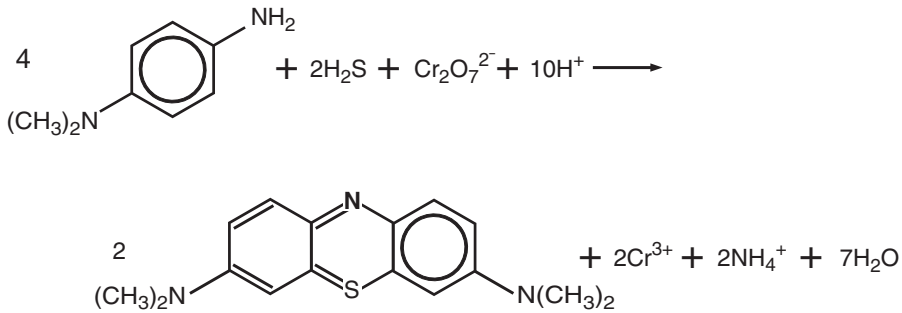


Figure 92 Chemical reaction for hydrogen sulfide using the Methylene Blue method

Sulfite

For water, wastewater and seawater **Titration Method**

Introduction

Sulfite is most commonly found in boilers and boiler feedwater, where it is used to inhibit corrosion by reducing dissolved oxygen. It may also be found in industrial wastes such as paper mill effluents. Sulfite normally is not present in natural waters because it readily oxidizes to sulfate.

Chemical reactions

The water sample is acidified by the addition of Dissolved Oxygen 3 Reagent Powder Pillow, starch indicator is then added and the solution is titrated with Potassium Iodide-Iodate Solution. The acidified solution releases free iodine, which oxidizes sulfite to form sulfate. The iodine is reduced to colorless iodide:

$KIO_3 + 5KI + 6HCl \rightarrow 6KCl + 3I_2 + 3H_2O$

$SO_3^{2-} + I_2 + H_2O \rightarrow SO_4^{2-} + 2HI$

When all sulfite has been converted to sulfate, excess iodine is indicated by a blue color from the starch-iodine reaction.

Turbidity

Introduction and definition

Turbidity, a qualitative characteristic which is imparted by solids obstructing the transmittance of light through a water sample, is an important water quality indicator. Turbidity can be interpreted as a measure of the relative clarity of water and often indicates the presence of dispersed, suspended solids; particles not in true solution such as silt, clay, algae and other microorganisms; organic matter and other minute particles. Turbidity is not a direct measure of suspended particles in water, but a measure of the scattering effect such particles have on light.

The extent to which suspended solids can be tolerated varies widely, as do the levels at which they exist. Industrial cooling water, for example, can tolerate relatively high levels of suspended solids without significant problems. In modern high pressure boilers, however, water must be virtually free of impurities. Solids in drinking water can support growth of harmful microorganisms and reduce effectiveness of chlorination, resulting in health hazards. In almost all water supplies, high levels of suspended matter are unacceptable for aesthetic reasons and can interfere with chemical and biological tests.

Theory of light scattering

Very simply, the optical property expressed as turbidity is the interaction between light and suspended particles in water. A directed beam of light remains relatively undisturbed when transmitted through absolutely pure water, but even the molecules in a pure fluid will scatter light to a certain degree. Therefore, no solution will have a zero turbidity. In samples containing suspended solids, the manner in which the sample interferes with light transmittance is related to the size, shape and composition of the particles in the solution and to the wavelength (color) of the incident light.

A minute particle interacts with incident light by absorbing the light energy and then, as if a point light source itself, reradiating the light energy in all directions. This omnidirectional reradiation constitutes the "scattering" of the incident light. The spatial distribution of scattered light depends on the ratio of particle size to wavelength of incident light. Particles much smaller than the wavelength of incident light exhibit a fairly symmetrical scattering distribution with approximately equal amounts of light scattered both forward and backward (see Figure 93).

As particle sizes increase in relation to wavelength, light scattered from different points of the sample particle create interference patterns that are additive in the forward direction. This constructive interference results in forward-scattered light of a higher intensity than light scattered in other directions (see Figure 94 and Figure 95). In addition, smaller particles scatter shorter (blue) wavelengths more intensely while having little effect on longer (red) wavelengths. Conversely, larger particles scatter long wavelengths more readily than they scatter short wavelengths of light.

Particle shape and refractive index also affect scatter distribution and intensity. Spherical particles exhibit a larger forward-to-back scatter ratio than coiled or rod-shaped particles. The refractive index of a particle is a measure of how it redirects light passing through it from another medium such as the suspending fluid. The particle's refractive index must be different than the refractive index of the sample fluid in order for scattering to occur. As the difference between the refractive indices of suspended particle and suspending fluid increases, scattering become more intense.

The color of suspended solids and sample fluid are significant in scattered-light detection. A colored substance absorbs light energy in certain bands of the visible spectrum, changing the character of both transmitted light and scattered light and preventing a certain portion of the scattered light from reaching the detection system.

Light scattering intensifies as particle concentration increases. But as scattered light strikes more and more particles, multiple scattering occurs and absorption of light increases. When particulate concentration exceeds a certain point, detectable levels of both scattered and transmitted light

drop rapidly, marking the upper limit of measurable turbidity. Decreasing the path length of light through the sample reduces the number of particles between the light source and the light detector and extends the upper limit of turbidity measurement.

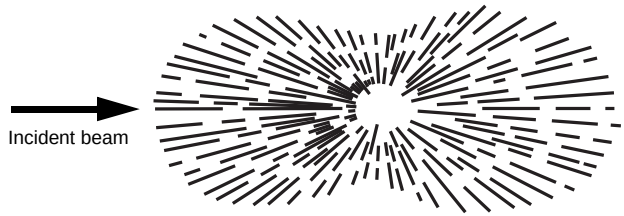


Figure 93 Effects of particle size and light wavelength (small particles)[1]

[1] Size: Smaller Than 1/10 the wavelength of light
Description: Scattering symmetric

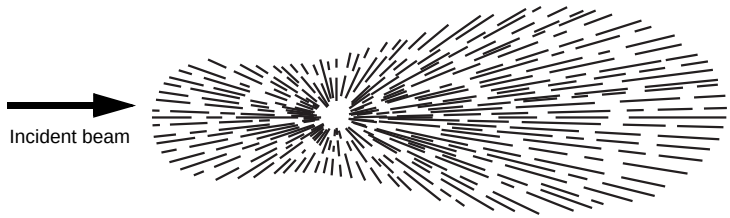


Figure 94 Effects of particle size and light wavelength (large particles)[1]

[1] Size: Approximately 1/4 the wavelength of light
Description: Scattering concentrated in forward direction

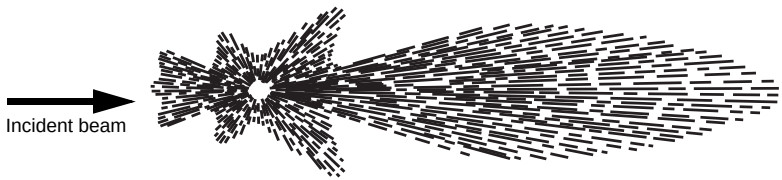


Figure 95 Effects of particle size and light wavelength (larger particles)[1]

[1] Size: Larger Than the wavelength of light
Description: Extreme concentration of scattering in forward direction;
Development of Maxima and Minima of scattering Intensity at wider angles

General instrument description

The instrument optical system typically includes a lamp, lenses and apertures to focus the light, a 90-degree detector to monitor scattered light and optionally, a forward-scatter light detector, a transmitted-light detector and a back-scatter light detector. The optional detectors may be added to minimize the impact of color, stray light and lamp and optical variabilities (see Figure 96).

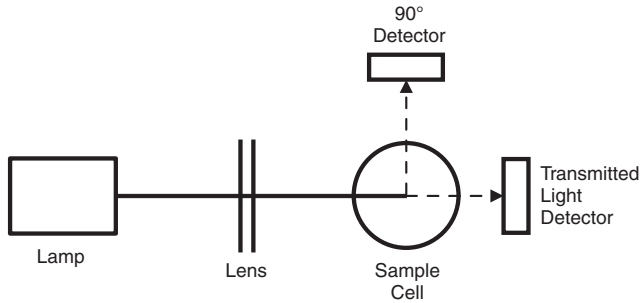


Figure 96 General turbidimeter optical system

Formazin primary standard

The chemically accepted definition of a primary standard is a standard that is prepared by the end user, on the bench, from traceable raw materials.

Formazin meets that criteria when prepared by accurately weighing and dissolving 5.000 g of hydrazine sulfate and 50.0 g of hexamethylenetetramine in one liter of distilled water. The solution develops a white turbidity after standing at 25 °C (77 °F) for 48 hours and can be prepared repeatably with an accuracy of ±1%. This solution is equal to 4000 NTU. All other turbidity standards are traced to formazin.

Due to the statistical reproducibility of the nephelometric scatter of white light by the formazin polymer, instruments with the traditional tungsten filament white light optical designs can be calibrated with a high degree of accuracy and reproducibility. The randomness of particle shapes and sizes within formazin standards yield statistically reproducible scatter on all makes and models of turbidimeters.

Zinc

For water and wastewater **Zincon Method**

Introduction

Zinc concentrations in most water supplies average about 1 mg/L, but may range as high as 50 mg/L in some areas. Although zinc is commonly found in many natural waters, the deterioration of galvanized iron and leaching of brass can add substantial amounts. Industrial effluents may contribute large amounts of zinc; high concentrations suggest the presence of lead and cadmium, both common impurities from the galvanizing process.

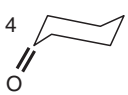
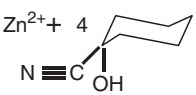
Zinc is essential to human metabolism and has been found to be necessary for proper growth. High concentrations of zinc in water act as stomach irritants but the effects are temporary. Concentrations above 5 mg/L show no harmful physiological effects but can cause a bitter taste and/or an opalescence in alkaline drinking water.

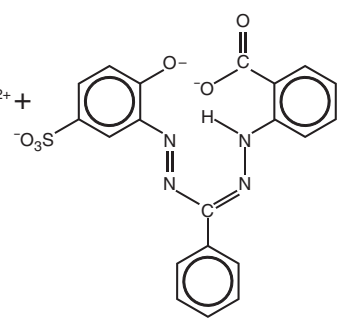
A dry powder form of 2-carboxy-2'hydroxy-5'sulfoformazyl benzene indicator, commonly called zincon, is used in the ZincoVer® Method of determining zinc concentrations. This test has been approved by the Environmental Protection Agency for National Pollutant Discharge Elimination System-reporting purposes based on comparability studies if the sample is first digested. Testing done for non-reporting purposes generally does not require sample digestion.

Chemical reaction

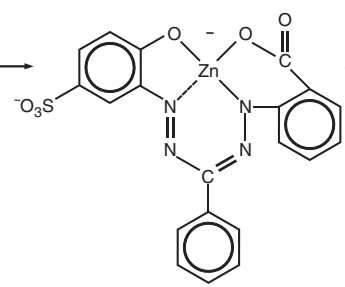
In the analysis of zinc, cyanide is added to a buffered water sample of pH 9 to form a complex with all heavy metals present in the sample. (1) The addition of cyclohexanone then frees the zinc from the cyanide complex (2) and enables it to react with the indicator, zincon (3) a blue-colored complex forms in direct proportion to the amount of zinc in the sample. Measurement of the color intensity determines the zinc concentration.

(1) $Zn^{2+} + 4CN^- \longrightarrow Zn(CN)_4^{2-}$

(2) $Zn(CN)_4^{2-} + 4$  $+ 4H_2 \longrightarrow Zn^{2+} + 4$  $+ 4OH^-$

(3) $^-OH + Zn^{2+} +$ 

Zincon (orange)



(Blue)

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