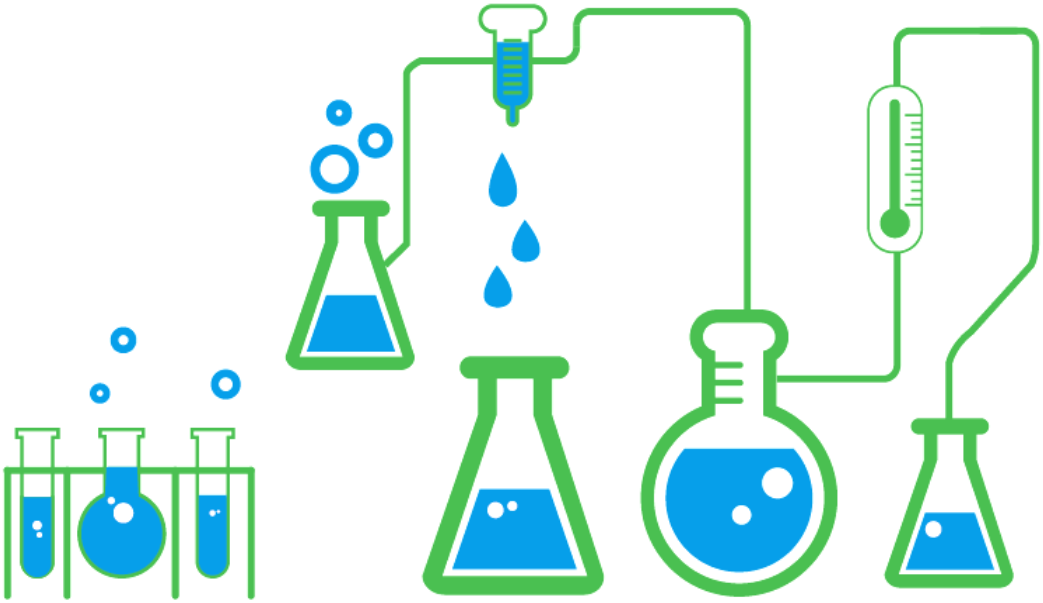
Water Treatment Laboratory Workshop

Week 1

Course # 2112



Fleming Training Center
Your Partner in Clean Water

TN Department of Environment & Conservation

Updated March 2022

Water Treatment Laboratory
Week 1
Course # 2122
March 14-18, 2022

Instructor: Amanda Carter

Monday

8:30	Basic Lab Skills/Safety/Lab Equipment
11:00	Lunch
12:15	QA/QC

Tuesday

8:30	pH
9:30	Specific Gravity
11:00	Lunch
12:00	Potassium Permanganate Residual
1:00	Chlorine, Free Residual & Total

Wednesday

8:30	Bacteriological Test
11:00	Lunch
12:00	Tour - to be announced

Thursday

8:30	Turbidity
10:30	Jar Testing
11:30	Lunch
12:30	Corrosion Control

Friday

8:30	Lead & Copper
9:00	Review/Questions
11:00	Lunch
12:00	Class Exam and Evaluations

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Water Treatment Laboratory
Week 1
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Lab Policies

1. No horse play.
2. No shorts or open-toed shoes.
3. No smoking, eating, dipping or drinking in the lab.
4. Put broken glass in broken glass container, NOT IN THE TRASH.
5. Do not pipet by mouth.
6. Each day after class:
    - ♦ All used glassware will be washed in hot soapy water, rinsed in tap water, then distilled water.
    - ♦ All counter tops will be wiped clean with disinfectant.
    - ♦ Balance room must be clean.
7. Used pipets are placed in containers containing detergent immediately after use, tip up.
8. Acid spills must be cleaned up immediately.
9. Pipet bulbs must be cleaned immediately after overpipeting.
10. Wear safety glasses when performing any experiment.
11. Wear aprons in the lab at all times.
12. Wear gloves when performing any experiment or washing glassware.
13. Wash your hands before leaving the laboratory.
14. Know where the eye wash stations are located and how to use them.
15. Know where the emergency shower is and how to use it.
16. Know where each fire extinguisher is located and how to use them.
17. Read carefully the Safety Data Sheets for all chemicals used in the laboratory.

Periodic Table of the Elements

KEY

Atomic Mass → 12.011 ← Selected Oxidation States: −4, +2, +4

Symbol → **C**

Atomic Number → **6**

Electron Configuration → 2-4

Relative atomic masses are based on $^{12}C = 12.000$

Note: Mass numbers in parentheses are mass numbers of the most stable or common isotope.

Period	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10	Group 11	Group 12	Group 13	Group 14	Group 15	Group 16	Group 17	Group 18
1	1.00794 +1 −1 **H** **1** 1																	4.00260 0 **He** **2** 2
2	6.941 +1 **Li** **3** 2-1	9.01218 +2 **Be** **4** 2-2											10.81 +3 **B** **5** 2-3	12.0111 −4 +2 +4 **C** **6** 2-4	14.0067 −3 −2 −1 +1 +2 +3 +4 +5 **N** **7** 2-5	15.9994 −2 **O** **8** 2-6	18.998403 −1 **F** **9** 2-7	20.179 0 **Ne** **10** 2-8
3	22.98977 +1 **Na** **11** 2-8-1	24.305 +2 **Mg** **12** 2-8-2											26.98154 +3 **Al** **13** 2-8-3	28.0855 −4 +2 +4 **Si** **14** 2-8-4	30.97376 −3 +3 +5 **P** **15** 2-8-5	32.06 −2 +4 +6 **S** **16** 2-8-6	35.453 −1 +1 +3 +5 +7 **Cl** **17** 2-8-7	39.948 0 **Ar** **18** 2-8-8
4	39.0983 +1 **K** **19** 2-8-8-1	40.08 +2 **Ca** **20** 2-8-8-2	44.9559 +3 **Sc** **21** 2-8-9-2	47.88 +2 +3 +4 **Ti** **22** 2-8-10-2	50.9415 +2 +3 +4 +5 **V** **23** 2-8-11-2	51.996 +2 +3 +6 **Cr** **24** 2-8-13-1	54.9380 +2 +3 +4 +7 **Mn** **25** 2-8-13-2	55.847 +2 +3 **Fe** **26** 2-8-14-2	58.9332 +2 +3 **Co** **27** 2-8-15-2	58.69 +2 +3 **Ni** **28** 2-8-16-2	63.546 +1 +2 **Cu** **29** 2-8-18-1	65.39 +2 **Zn** **30** 2-8-18-2	69.72 +3 **Ga** **31** 2-8-18-3	72.59 −4 +2 +4 **Ge** **32** 2-8-18-4	74.9216 −3 +3 +5 **As** **33** 2-8-18-5	78.96 −2 +4 +6 **Se** **34** 2-8-18-6	79.904 −1 +1 +5 **Br** **35** 2-8-18-7	83.80 0 +2 **Kr** **36** 2-8-18-8
5	85.4678 +1 **Rb** **37** 2-8-18-8-1	87.62 +2 **Sr** **38** 2-8-18-8-2	88.9059 +3 **Y** **39** 2-8-18-9-2	91.224 +4 **Zr** **40** 2-8-18-10-2	92.9064 +3 +5 **Nb** **41** 2-8-18-12-1	95.94 +3 +6 **Mo** **42** 2-8-18-13-1	(98) +4 +6 +7 **Tc** **43** 2-8-18-14-1	101.07 +3 **Ru** **44** 2-8-18-15-1	102.906 +3 **Rh** **45** 2-8-18-16-1	106.42 +2 +4 **Pd** **46** 2-8-18-18	107.868 +1 **Ag** **47** 2-8-18-18-1	112.41 +2 **Cd** **48** 2-8-18-18-2	114.82 +3 **In** **49** 2-8-18-18-3	118.71 +2 +4 **Sn** **50** 2-8-18-18-4	121.75 −3 +3 +5 **Sb** **51** 2-8-18-18-5	127.60 −2 +4 +6 **Te** **52** 2-8-18-18-6	126.905 −1 +1 +5 +7 **I** **53** 2-8-18-18-7	131.29 0 +2 +4 +6 **Xe** **54** 2-8-18-18-8
6	132.905 +1 **Cs** **55** 2-8-18-18-8-1	137.33 +2 **Ba** **56** 2-8-18-18-8-2	138.906 +3 **La** **57** 2-8-18-18-9-2	178.49 +4 **Hf** **72** **18-32-10-2	180.948 +5 **Ta** **73** -18-32-11-2	183.85 +6 **W** **74** -18-32-12-2	186.207 +4 +6 +7 **Re** **75** -18-32-13-2	190.2 +3 +4 **Os** **76** -18-32-14-2	192.22 +3 +4 **Ir** **77** -18-32-15-2	195.08 +2 +4 **Pt** **78** -18-32-17-1	196.967 +1 +3 **Au** **79** -18-32-18-1	200.59 +1 +2 **Hg** **80** -18-32-18-2	204.383 +1 +3 **Tl** **81** -18-32-18-3	207.2 +2 +4 **Pb** **82** -18-32-18-4	208.980 +3 +5 **Bi** **83** -18-32-18-5	(209) +2 +4 **Po** **84** -18-32-18-6	(210) **At** **85** -18-32-18-7	(222) 0 **Rn** **86** -18-32-18-8
7	(223) +1 **Fr** **87** -18-32-18-8-1	226.025 +2 **Ra** **88** -18-32-18-8-2	227.028 +3 **Ac** **89** -18-32-18-9-2	(261) **Rf** **104**	(262) **Db** **105**	(263) **Sg** **106**	(264) **Bh** **107**	(265) **Hs** **108**	(268) **Mt** **109**	(269) **Uun**	(272) **Uuu** **111**	(277) **Uub** **112**		(285) **Uuq** **114**					

140.12 +3 +4 **Ce** **58**	140.908 +3 **Pr** **59**	144.24 +3 **Nd** **60**	(145) +3 **Pm** **61**	150.36 +2 +3 **Sm** **62**	151.96 +2 +3 **Eu** **63**	157.25 +3 **Gd** **64**	158.925 +3 **Tb** **65**	162.50 +3 **Dy** **66**	164.930 +3 **Ho** **67**	167.26 +3 **Er** **68**	168.934 +3 **Tm** **69**	173.04 +2 +3 **Yb** **70**	174.967 +3 **Lu** **71**
232.038 +4 **Th** **90**	231.036 +4 +5 **Pa** **91**	238.029 +3 +4 +5 +6 **U** **92**	237.048 +3 +4 +5 +6 **Np** **93**	(244) +3 +4 +5 +6 **Pu** **94**	(243) +3 +4 +5 +6 **Am** **95**	(247) +3 **Cm** **96**	(247) +3 +4 **Bk** **97**	(251) +3 **Cf** **98**	(252) **Es** **99**	(257) **Fm** **100**	(258) **Md** **101**	(259) **No** **102**	(260) **Lr** **103**

**Denotes the presence of (2-8-) for elements 72 and above

*The systematic names and symbols for elements of atomic numbers above 109 will be used until the approval of trivial names by IUPAC.

Common Valences

1+	*2+*	*3+*
Ammonium, NH_4^+	Barium, Ba^{2+}	Aluminum, Al^{3+}
Cuprous, Cu^+	Calcium, Ca^{2+}	Chromic, Cr^{3+}
Hydrogen, H^+	Cupric, Cu^{2+}	Ferric, Fe^{3+}
Hydronium, H_3O^+	Ferrous, Fe^{2+}	
Potassium, K^+	Lead, Pb^{2+}	
Silver, Ag^+	Magnesium, Mg^{2+}	
Sodium, Na^+	Mercuric, Hg^{2+}	
	Nickel, Ni^{2+}	
	Zinc, Zn^{2+}	

1-	*2-*	*3-*
Bicarbonate, HCO_3^-	Carbonate, CO_3^{2-}	Phosphate, PO_4^{3-}
Bromide, Br^-	Chromate, CrO_4^{2-}	
Chlorate, ClO_3^-	Peroxide, O_2^{2-}	
Chloride, Cl^-	Sulfate, SO_4^{2-}	
Hydroxide, OH^-	Sulfide, S^{2-}	
Hypochlorite, OCl^-	Sulfite, SO_3^{2-}	
Iodide, I^-		
Nitrate, NO_3^-		
Nitrite, NO_2^-		
Bisulfate, HSO_4^-		
Permanganate, MnO_4^-		

How many grams of Na_2CO_3 would it take to make a 1 Molar and a 1 Normal solution?

Step 1: Determine the molecular weight of Na_2CO_3.

$Na_2CO_3 \rightarrow Na^+ + Na^+ + CO_3^{2-}$

Na = 22.99 amu X 2 = 45.98 amu
C = 12.01 amu X 1 = 12.01 amu
O = 16 amu X 3 = 48.00 amu
Molecular weight = 105.99 amu

Molarity

Step 2: Calculate the number of moles

$$\#\text{ moles} = \frac{\text{total weight}}{\text{molecular weight}}$$

$$\#\text{ moles} = \frac{105.99\text{ g}}{105.99\text{ g/mole}}$$

$$\#\text{ moles} = 1$$

or

grams required = (#moles required)(grams/mole)
grams required = (1 mole)(105.99 grams/mole)
grams required = 105.99 g

Step 3: Calculate the Molarity of the solution.

$$\text{Molarity} = \frac{\#\text{ of moles}}{\text{volume of solvent}}$$

$$\text{Molarity} = \frac{1\text{ mole}}{1\text{ liter of water}}$$

Molarity = 1M

Every 105.99 grams of Na_2CO_3 in 1 L of water gives a 1M solution.

Normality

Step 2a: Calculate the equivalent weight.

$$\text{equivalent weight} = \frac{\text{molecular weight}}{\#\text{ of positive charges}}$$

$$\text{equivalent weights} = \frac{105.99}{2}$$

equivalent weights = 53 grams

Step 2b: Calculate the number of equivalent weights.

$$\#\text{ equivalent weights} = \frac{\text{total weight}}{\text{equivalent weight}}$$

$$\#\text{ equivalent weights} = \frac{105.99\text{ g}}{53\text{ g/equivalent}}$$

$$\#\text{ equivalent weights} = 2$$

or

grams required = (# equivalent weights)(grams/equivalent weight)
grams required = (2 equivalent weights)(53 g/equivalent)
grams required = 106 g

Step 3: Calculate Normality

$$\text{Normality} = \frac{\#\text{ of equivalents}}{\text{volume of solvent}}$$

$$\text{Normality} = \frac{2\text{ equivalents}}{1\text{ liter of water}}$$

Normality = 2N

Every 105.99 g of Na_2CO_3 in 1 L of water gives a 2N solution. To make a 1N solution, cut the grams in half.

Laboratory Safety Practices

OSHA's Hazard Communication Standard (HCS)

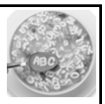
What is HCS?

- Employers that have hazardous chemicals in their workplaces are required by OSHA's Hazard Communication Standard (HCS), 29 CFR 1910.1200, to implement a hazard communication program.
- The program must include labels on containers of hazardous chemicals, safety data sheets (SDSs) for hazardous chemicals, and training for workers.
- Each employer must also describe in a written program how it will meet the requirements of the HCS in each of these areas.

OSHA's Hazard Communication Standard (HCS)

- OSHA's Hazard Communication Standard (HCS) is designed to protect against chemical-source injuries and illnesses by ensuring that employers and workers are provided with sufficient information to anticipate, recognize, evaluate, and control chemical hazards and take appropriate protective measures.
  - "Right-to-Know" standard
- This information is provided through safety data sheets (SDSs), labels, and employee training.

OSHA HCS GHS



- OSHA revised the Hazard Communication Standard (HCS) to align with the United Nation's Globally Harmonized System (GHS)
- Changed standard from a performance-based standard to one that has more structured requirements for the labeling of chemicals.
- Revised criteria for chemical hazard classification, labeling & new format for Safety Data Sheets (SDS).
  - Safety Data Sheet must be maintained on file for 30 years after chemical disposal.

Safety Data Sheet (SDS)

- Must be provided by chemical manufacturer, distributor, or importer
- Formerly known as MSDS
  - Now has consistent user-friendly format
- Sections 1-8 contain general information about the chemical
- Sections 9-11 & 16 contain other technical & scientific information
- Sections 12-15 are not enforced by OSHA, but must be present to be in compliance with GHS

Safety Data Sheet Required Format

1. Product identification
2. Hazard Identification
3. Information on ingredients
4. First-aid measures
5. Fire-fighting measures
6. Accidental release measures
7. Handling and storage
8. Exposure controls
9. Physical/chemical properties
10. Stability & reactivity
11. Toxicological information
12. Ecological information*
13. Disposal considerations*
14. Transport information*
15. Regulatory information*
16. Other information (including date of SDS or last revision)

* Non-mandatory

OSHA Chemical Labels

- Labels for hazardous chemicals must include:
  - Name, Address and Telephone Number
    - Chemical manufacturer, importer or other responsible party
  - Product Identifier
    - How the hazardous chemical is identified.
    - Same product identifier must be on the label and SDS
  - Signal Word
    - Used to indicate the relative level of severity of the hazard
    - "Danger" is used for the more severe hazards
    - "Warning" is used for the less severe hazards

OSHA Chemical Labels

- Labels for hazardous chemicals must include:
  - Hazard Statement(s)
    - Describes the nature of the hazard(s) of a chemical, including the degree of hazard.
    - Example: "Causes damage to kidneys through prolonged or repeated exposure when absorbed through the skin."
    - All of the applicable hazard statements must appear on the label.

OSHA Chemical Labels

- Labels for hazardous chemicals must include:
  - Precautionary Statement(s)
    - Recommended measures that should be taken to minimize or prevent adverse effects
    - There are four types of precautionary statements:
      - Prevention - to minimize exposure
      - Response - in case of accidental spillage or exposure, emergency response, & first-aid
      - Storage
      - Disposal
  - Pictogram(s)

OSHA Pictograms

- Appendix C, Section C.2.3.1 of 29 CFR 1910.1200 states the following:
  - Pictograms shall be in the shape of a square set at a point and shall include a black hazard symbol on a white background with a red frame sufficiently wide to be clearly visible.
  - **A square red frame set at a point without a hazard symbol is not a pictogram and is not permitted on the label.**

OSHA Pictograms

*Environment


Flame Over Circle


Flame


Exploding Bomb


Skull & Crossbones

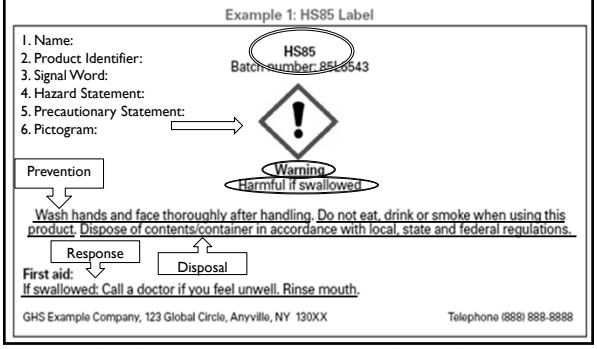
Corrosion

Gas Cylinder

Health Hazard

Exclamation Mark

Example: HCS Label



Example: HCS Label

- Name: HS85 Batch #: 85L6543
- Product Identifier: HS85 Batch #: 85L6543
- Signal Word: WARNING
- Hazard Statement: Harmful if swallowed
- Precautionary Statement: Wash hands and face thoroughly after handling. Do not eat, drink or smoke when using this product. Dispose of contents/container in accordance with local, state, and federal regulations.
  - First Aid: If swallowed: call a doctor if you feel unwell. Rinse mouth.
- Pictogram:  Irritant

HCS Labels

- Purpose:
  - Informs workers about the hazards of chemicals in workplace under normal conditions of use and foreseeable emergencies.
- Number System:
  - 1-4
  - 1 – most severe hazard ; 4 – lease severe hazard
- Information provided on label:

Product Identifier	Precautionary Statement
Signal Word	Pictogram
Hazard Statement	Contact information

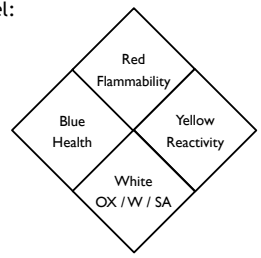
National Fire Protection Association (NFPA) 704 Label

- Purpose:
  - NFPA 704 label provides a simple, readily recognized, easily understood system for identifying the specific hazards of a material and the severity of the hazard that would occur during an emergency response.
  - Addresses the health, flammability, instability, and special hazards presented from short-term, acute exposures that could occur as a result of a emergency.

National Fire Protection Association (NFPA) 704 Label

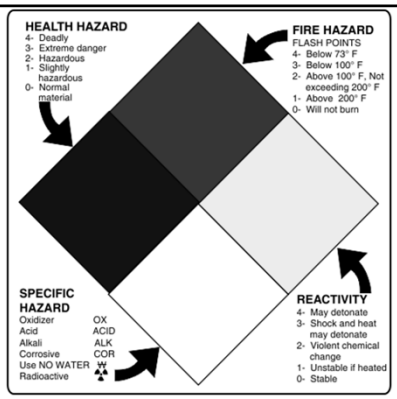
- Number rating system:
  - 0-4; 0 – least hazardous; 4 – most hazardous
- Information provided on label:
  - Health
  - Flammability
  - Reactivity
  - Special Hazards

OX ⟶ oxidizer
~~W~~ ⟶ use NO WATER
SA ⟶ simple asphyxiant



	 NFPA 704 Label	 HazCom2012
Purpose	Provides information about hazards that occur during emergency response.	Provides information about hazards to workers using chemicals under normal conditions of Use.
Label Location	Pipes, drums, and containers of materials that are used in the Workplace.	Outside buildings, on doors, on tanks, visible to emergency responders during spill or fire
Health Hazards	Acute (short term) health hazards ONLY.	Acute (short term) and chronic (long term) health hazards.
Flammability/Physical Hazards	NFPA divides flammability and instability hazards into two separate numbers on the label.	A broad range of physical hazard classes are listed on the label …

NFPA 704 LABEL



Labels on Portable/Secondary Containers

- Portable containers must comply with the labeling requirements listed above if any of the following events occur:
  - The material is not used within the work shift of the individual who makes the transfer.
  - The worker who made the transfer leaves the work area.
  - The container is moved to another work area and is no longer in the possession of the worker who filled the container.
- Labels on portable containers are not required if the worker who made the transfer uses all of the contents during the work shift.

Labels on Portable/Secondary Containers

- If a secondary container that does not meet Permenant Label requirements is used for more than one shift, a label must be applied to the secondary container.
- Secondary container label must contain 2 key pieces of information:
  - Identity of the hazardous chemical in the container
    - e.g., chemical name
  - Greatest hazard present in chemical

Replacement Container Labels

- The existing label on a container entering the workplace from a supplier must not be removed, altered or defaced.
- If a chemical container's original label must be replaced, the new label must contain the same information as the original
- Only use labels, inks and markings that are not soluble in the liquid content of the container.

Hazard Communication Standard (HCS) (29 CFR 1910.1200)

- The steps that employers must take to comply with the requirements of this standard must include, but are not limited to:
  - Development and maintenance of a written hazard communication program for the workplace, including lists of hazardous chemicals present;
  - Ensuring that containers of chemicals in the workplace … are properly labeled;
  - Ensuring that safety data sheets (SDSs) for chemicals that workers may be exposed to are made available to workers; and
  - Development and implementation of worker training programs regarding hazards of chemicals they may be exposed to and the appropriate protective measures that must be used when handling these chemicals.

Laboratory Safety Guidance

OSHA 3404-11R 2011

Fleming Training Center | TN Department of Environment & Conservation

OSHA Standards

- Section 5(a)(1) of the Occupational Safety and Health Act of 1970 (OSH Act), the General Duty Clause, requires that employers "shall furnish to each of his employees' employment and a place of employment which are free from recognized hazards that are causing or likely to cause death or serious physical harm to his employees."

OSHA Standards for Non-Production Laboratories

- ★ Occupational Exposure to Hazardous Chemicals in Laboratories standard (29 CFR 1910.1450)
- ★ Hazard Communication standard (29 CFR 1910.1200)
- Bloodborne Pathogens standard (29 CFR 1910.1030)
- ★ Personal Protective Equipment (PPE) standard (29 CFR 1910.132)
- Eye and Face Protection standard (29 CFR 1910.133)
- Respiratory Protection standard (29 CFR 1910.134)
- ★ Hand Protection standard (29 CFR 1910.138)
- The Control of Hazardous Energy standard (29 CFR 1910.147)

Hierarchy of Controls

- Prioritizes intervention strategies based on the premise that the best way to control a hazard is to systematically remove it from the workplace, rather than relying on workers to reduce their exposure.
- The types of measures that may be used to protect laboratory workers, prioritized from the most effective to least effective, are:
  - Engineering controls
  - Administrative controls
  - Work practices
  - Personal protective equipment (PPE)

Hierarchy of Controls

- Engineering Controls
  - Those measures that involve making changes to the work environment to reduce work-related hazards.
  - Preferred over all others because they make permanent changes that reduce exposure to hazards and do not rely on worker behavior.
  - Can be the most cost-effective solutions for employers to implement by reducing a hazard in the workplace.
  - Examples include:
    - Chemical Fume Hoods
    - Biological Safety Cabinets (BSCs).

Hierarchy of Controls

- Administrative Controls
  - Those controls that modify workers' work schedules and tasks in ways that minimize their exposure to workplace hazards.
  - Examples include:
    - Developing a Chemical Hygiene Plan; and
    - Developing Standard Operating Procedures for chemical handling
- Work practices
  - Procedures for safe and proper work that are used to reduce the duration, frequency or intensity of exposure to a hazard.
  - Examples include:
    - No mouth pipetting
    - Chemical substitution where feasible

Hierarchy of Controls

- Personal Protective Equipment (PPE)
  - Protective gear needed to keep workers safe while performing their jobs.
  - It is important that PPE be:
    - Selected based upon the hazard to the worker;
    - Properly fitted and in some cases periodically refitted;
    - Conscientiously and properly worn;
    - Regularly maintained and replaced according to manufacturer;
    - Properly removed and disposed of to avoid contamination of self, others or the environment; and
    - If reusable, properly removed, cleaned, disinfected and stored

Chemical Hazards

- Laboratory chemicals include carcinogens, toxins, irritants, corrosives, sensitizers, as well as agents that act on the blood system or damage the lungs, skin, eyes, or mucous membranes.
- In 1990, OSHA issued the Occupational Exposure to Hazardous Chemicals in Laboratories standard (29 CFR 1910.1450).
  - Commonly known as the Laboratory standard
  - Developed to address workplaces where relatively small quantities of hazardous chemicals are used on a non-production basis

Laboratory Standard (29 CFR 1910.1450)

- Occupational Exposure to Hazardous Chemicals in Laboratories standard (29 CFR 1910.1450)
  - Created specifically for non-production laboratories
- Laboratory workers are exposed to numerous potential hazards including chemical, biological, physical and radioactive hazards, as well as musculoskeletal stresses.
- Worker guidance in the form of Fact Sheets and QuickCards™ is also provided for certain hazards that may be encountered in laboratories.

Laboratory Standard (29 CFR 1910.1450)

- Developed to address workplaces where relatively small quantities of hazardous chemicals are used on a non-production basis.
- Purpose is to ensure that workers in non-production laboratories are informed about the hazards of chemicals in their workplace and are protected from chemical exposures exceeding allowable levels.
  - i.e., OSHA permissible exposure limits (PELs)
- Establishes safe work practices in laboratories to implement a Chemical Hygiene Plan (CHP).

Laboratory Standard (29 CFR 1910.1450)

Scope and Application

- …applies to all individuals engaged in laboratory use of hazardous chemicals.

Program Description

- The Laboratory standard consists of five major elements:
  - Hazard identification
  - Chemical Hygiene Plan
  - Information and training
  - Exposure monitoring
  - Medical consultation and examinations

Laboratory Standard (29 CFR 1910.1450)

Hazard Identification

- Each laboratory must identify which hazardous chemicals will be encountered by its workers.
- **All** containers for chemicals must be clearly labeled.
- Labels must not be removed or defaced.
- Safety Data Sheets (SDSs) for chemicals received by must be maintained and readily accessible to laboratory workers.
- Employers must have an SDS in the workplace for each hazardous chemical in use.

Laboratory Standard (29 CFR 1910.1450)

Chemical Hygiene Plan (CHP)

- The purpose of the CHP is to provide guidelines for prudent practices and procedures for the use of chemicals in the laboratory.

Laboratory Standard (29 CFR 1910.1450)

Chemical Hygiene Plan (CHP) must include:

- Standard Operating Procedures (SOPs)
- Criteria for Exposure Control Measures
- Adequacy and Proper Functioning of Fume Hoods and other Protective Equipment
- Information and Training
- Requirement for Prior Approval of Laboratory Procedures
- Medical Consultations and Examinations
- Chemical Hygiene Officer Designation
- Particularly Hazardous Substances

Laboratory Standard (29 CFR 1910.1450)

Information and Training

- The employer must inform workers about the following:
  - Content of the OSHA Laboratory standard & its appendices
  - Location & availability of the Chemical Hygiene Plan
  - Permissible exposure limits (PELs) for OSHA regulated substances…
  - Signs & symptoms associated with exposure to hazardous chemicals in the laboratory
  - Location and availability of reference materials on the hazards, safe handling, storage and disposal of hazardous chemicals in the laboratory, including, but not limited to, (M)SDSs.

Laboratory Standard (29 CFR 1910.1450)

Information and Training:

- Training must include the following:
  - Methods and observations used to detect the presence or release of a hazardous chemical.
  - The physical and health hazards of chemicals in the laboratory work area;
  - The measures that workers can take to protect themselves from these hazards, …;
  - Applicable details of the employer's written Chemical Hygiene Plan;
  - Retraining, if necessary.

Laboratory Standard (29 CFR 1910.1450)

- Permissible Exposure Limits (PELs) are the chemical-specific concentrations in inhaled air that is intended to represent what the average, healthy worker may be exposed to daily for a lifetime of work without significant adverse health effects.
  - The employer must ensure that workers' exposures to OSHA-regulated substances do not exceed the PEL.
  - Employers must conduct exposure monitoring, through air sampling, if there is reason to believe that workers may be exposed to chemicals above the action level or, in the absence of an action level, the PEL.

Laboratory Standard (29 CFR 1910.1450)

Medical Consultations and Examinations

- Employers must do the following:
  - Provide all exposed workers with an opportunity to receive medical attention by a licensed physician …
  - Establish medical surveillance for a worker as required … when exposure monitoring reveals exposure levels routinely exceeding the OSHA action level …

Laboratory Standard (29 CFR 1910.1450)

Medical Consultations and Examinations

- Employers must do the following:
  - Provide the examining physician with the identity of the hazardous chemical(s) to which the individual may have been exposed, and the conditions under which the exposure may have occurred, … and a description of the signs and symptoms of exposure the worker may be experiencing.
  - Provide all medical examinations and consultations without cost to the worker, without loss of pay, and at a reasonable time and place.
  - A copy of the examining physician's written opinion must be provided to the exposed worker.

Laboratory Standard (29 CFR 1910.1450)

Recordkeeping

- Employers must also maintain an accurate record of:
  - Exposure monitoring activities
  - Exposure measurements
  - Medical consultations and examinations
    - Including medical tests and written opinions.
- Employers generally must maintain worker exposure records for 30 years and medical records for the duration of the worker's employment plus 30 years.

Laboratory Standard (29 CFR 1910.1450)

Roles and Responsibilities in Implementing the Laboratory Standard

- Chief Executive Officer
  - Holds responsibility for chemical hygiene within the facility.
  - Provides continuing support for institutional chemical hygiene.
- Chemical Hygiene Officer
  - Develops & implements chemical hygiene policies and practices.
  - Monitors procurement, use, & disposal of lab chemicals.
  - Ensures appropriate audits are maintained.
  - Helps directors develop precautions & adequate facilities.
  - Knows the current legal requirements concerning regulated substances.
  - Seeks ways to improve the chemical hygiene program.

Laboratory Standard (29 CFR 1910.1450)

Roles and Responsibilities in Implementing the Laboratory Standard

- Laboratory Supervisors
  - Responsible for overall chemical hygiene in the laboratory.
  - Ensure that lab workers know & follow the chemical hygiene rules.
  - Ensure that protective equipment is available & in working order.
  - Ensure that appropriate training has been provided.
  - Provide regular, formal chemical hygiene & housekeeping inspections…
  - Know current legal requirements concerning regulated substances.
  - Determine the required levels of PPE and equipment.
  - Ensure that facilities and training for use of any material being ordered are adequate.

Laboratory Standard (29 CFR 1910.1450)

Roles and Responsibilities in Implementing the Laboratory Standard

- Laboratory Workers
  - Plan & conduct each operation in accordance with the facility's chemical hygiene procedures, including use of PPE and engineering controls, as appropriate.
  - Develop good personal chemical hygiene habits.
  - Report all accidents and potential chemical exposures immediately.

Biological Hazards (29 CFR 1910)

- Biological Agents (other than Bloodborne Pathogens) and Biological Toxins
  - Biological agents include bacteria, viruses, fungi, other microorganisms and their associated toxins.
  - They have the ability to adversely affect human health in a variety of ways, ranging from relatively mild, allergic reactions to serious medical conditions—even death.
  - Many laboratory workers encounter daily exposure to biological hazards.

Safety Hazards

- PPE Standard (29 CFR 1910.132)
  - Employers must assess tasks to identify potential worksite hazards and provide and ensure that workers use appropriate personal protective equipment (PPE)
- Hand Protection standard (29 CFR 1910.138)
  - Employers must require workers to use appropriate hand protection when hands are exposed to hazards such as sharp instruments and potential thermal burns.
  - Examples include using oven mitts when handling hot items…

Safety Hazards

- Autoclaves and Sterilizers
  - Workers should be trained to recognize the potential for exposure … when removing them from autoclaves/sterilizers …
  - In order to prevent injuries from occurring, employers must train workers to follow good work practices.

Safety Hazards - Electrical

- The potential for possible electrocution or electric shock or contact with electrical hazards can result from a number of factors, including the following:
  - Faulty electrical equipment/instrumentation or wiring
  - Damaged receptacles and connectors
  - Unsafe work practices

Safety Hazards - Fire

- Employers should ensure that workers are trained to do the following in order to prevent fires:
  - Plan work. Have a written emergency plan.
  - Minimize materials.
    - Have present in the immediate work area and use only the minimum quantities necessary for work in progress.
  - Observe proper housekeeping.
    - Keep work areas uncluttered, and clean frequently.
    - Put unneeded materials back in storage promptly.
    - Keep aisles, doors, and access to emergency equipment unobstructed at all times.
  - Observe restrictions on equipment.
  - Keep barriers in place (shields, hood doors, lab doors).
  - Wear proper clothing and personal protective equipment.

Safety Hazards - Fire

- Employers should ensure that workers are trained to do the following in order to prevent fires (continued):
  - Avoid working alone.
  - Store solvents properly in approved flammable liquid storage cabinets.
  - Shut door behind you when evacuating.
  - Limit open flames use to under fume hoods and only when constantly attended.
  - Keep combustibles away from open flames.
  - Do not heat solvents using hot plates.
  - Remember the “RACE” rule in case of a fire.
    - R= Rescue/remove all occupants
    - A= Activate the alarm system
    - C= Confine the fire by closing doors
    - E= Evacuate/Extinguish

Safety Hazards – Fire cont’d

- Employers should ensure that workers are trained in the following emergency procedures
  - Know what to do.
    - You tend to do under stress what you have practiced or pre-planned. Therefore, planning, practice and drills are essential.
  - Know where things are.
    - The nearest fire extinguisher, fire alarm box, exit(s), telephone, emergency shower/eyewash, and first-aid kit, etc.
  - Be aware that emergencies are rarely “clean” and will often involve more than one type of problem.

Safety Hazards – Fire cont’d

- Employers should train workers to remember the “PASS” rule for fire extinguishers
  - Train workers and exercise the emergency plan.
  - Learn to use the emergency equipment provided.
  - PASS summarizes the operation of a fire extinguisher.
    - P – Pull the pin
    - A – Aim extinguisher nozzle at the base of the fire
    - S – Squeeze the trigger while holding the extinguisher upright
    - S – Sweep the extinguisher from side to side; cover the fire with the spray

Safety Hazards – Fire cont'd

- Employers should train workers on appropriate procedures in the event of a clothing fire.
  - If the floor is not on fire, STOP, DROP and ROLL to extinguish the flames or use a fire blanket or a safety shower if there are no chemicals or electricity involved.
  - If a coworker's clothing catches fire and he/she runs down the hallway in panic, tackle him/her and smother the flames as quickly as possible, using appropriate means that are available (e.g., fire blanket, fire extinguisher).

Safety Hazards - Trips, Slips & Falls

- The Walking/Working Surfaces standard requires that all employers keep all places of employment clean and orderly and in a sanitary condition, 29 CFR 1910.22
- Keep floors clean and dry.
  - In addition to being a slip hazard, continually wet surfaces promote the growth of mold, fungi, and bacteria that can cause infections.
- Promote safe work practices, even in cramped working spaces.

Personal Protective Equipment (PPE)

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PPE Standard (29 CFR 1910.132)

- Protective equipment … shall be provided, used, and maintained in a sanitary and reliable condition wherever it is necessary …
- The employer shall assess the workplace to determine if hazards are present, or are likely to be present, which necessitate the use of personal protective equipment (PPE).

PPE Standard (29 CFR 1910.132)

- If such hazards are present, or likely to be present, the employer shall:
  - (i) Select, and have each affected employee use, the types of PPE that will protect the affected employee from the hazards identified in the hazard assessment;
  - (ii) Communicate selection decisions to each affected employee; and,
  - (iii) Select PPE that properly fits each affected employee.

PPE Standard (29 CFR 1910.132)

- The employer shall verify that the required workplace hazard assessment has been performed through a written certification that identifies the workplace evaluated; the person certifying that the evaluation has been performed; the date(s) of the hazard assessment; and, which identifies the document as a certification of hazard assessment.

PPE Standard (29 CFR 1910.132)

- The employer shall verify that the required workplace hazard assessment has been performed through a written certification that identifies the workplace evaluated; the person certifying that the evaluation has been performed; the date(s) of the hazard assessment; and, which identifies the document as a certification of hazard assessment.

PPE Standard (29 CFR 1910.132)

- The employer shall provide training to each employee who is required by this section to use PPE. Each such employee shall be trained to know at least the following:
  - When PPE is necessary;
  - What PPE is necessary;
  - How to properly don, doff, adjust, and wear PPE;
  - The limitations of the PPE; and,
  - The proper care, maintenance, useful life and disposal of the PPE.
- Each affected employee shall demonstrate an understanding of the training …, and the ability to use PPE properly, before being allowed to perform work requiring the use of PPE.

PPE Standard (29 CFR 1910.132)

- When the employer has reason to believe that any affected employee who has already been trained does not have the understanding and skill required … the employer shall retrain each such employee.
  - Circumstances where retraining is required include, but are not limited to, situations where:
    - Changes in the workplace render previous training obsolete;
    - Changes in the types of PPE to be used render previous training obsolete;
    - Inadequacies in an affected employee's knowledge or use of assigned PPE indicate that the employee has not retained the requisite understanding or skill.

PPE Standard (29 CFR 1910.132)

- ... personal protective equipment (PPE), used to comply with this part, shall be provided by the employer at no cost to employees.
- The employer is not required to pay for non-specialty safety-toe protective footwear (including steel-toe shoes or steel-toe boots) and non-specialty prescription safety eyewear, provided that the employer permits such items to be worn off the job-site.
- The employer is not required to pay for:
  - Everyday clothing, such as long-sleeve shirts, long pants, street shoes, and normal work boots; or
  - Ordinary clothing, skin creams, or other items, used solely for protection from weather, such as winter coats, jackets, gloves, parkas, rubber boots, hats, raincoats, ordinary sunglasses, and sunscreen.

PPE Standard (29 CFR 1910.132)

- The employer must pay for replacement PPE, except when the employee has lost or intentionally damaged the PPE.
- Where an employee provides adequate protective equipment, … the employer may allow the employee to use it and is not required to reimburse the employee for that equipment.
  - The employer shall not require an employee to provide or pay for his or her own PPE, unless the PPE is excepted [this rule].

Personal Protective Equipment

- Commonly referred to as “PPE”
- PPE is considered to be a last resort after the three more effective measures:
  - Engineering controls, administrative controls, & work practices.
- In a lab, common PPE items may include disposable gloves, thermoresistant gloves, safety glasses, safety goggles, face shield, apron, lab coat, earplugs or muffs, respirators, etc.

Personal Safety Practices

- Protective eyewear must be worn while performing tests
  - Safety glasses, safety goggles, etc.
- Lab coats and aprons should be worn while working in the lab
- Proper gloves are used as needed
  - Disposable, thermotolerant, etc.
- Other personal protective equipment used properly as needed
  - Respirator, protective footwear, etc.

Safety Glasses

- Unbreakable lenses of plastic or tempered glass
- For light-to-moderate work
- Do not interfere with contact lenses
- Cannot be worn with prescription glasses

Safety Goggles

- Work with significant risk of splash of chemicals or projectiles
- Can be worn over prescription glasses

Face Shield

- Use when working with significant risk of splash on face or possible explosion
- Some protect face adequately but not eyes
  - These should be worn with safety glasses and/or goggles to protect eyes

General Lab Safety Practices

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Personal Safety and Hygiene

- Never work alone in laboratory.
- Wear protective goggles or eyeglasses at all times in the laboratory.
  - If you wear prescription glasses, they will not suffice as eye protection unless they meet the same standards as the required safety goggles.
- Know location and operation of safety showers and eye wash stations before starting work
  - Should be inspected weekly

Personal Safety and Hygiene

- Never pipet hazardous liquids by mouth.
  - **Never put anything in your mouth while in the lab.**
- Always wear lab coat or apron and disposable gloves
- Wear insulated gloves when handling hot objects.
  - Wear face shield if there's a danger of hot liquid erupting.
- Do not bring food or drink into the lab or keep food in a refrigerator that is used for chemical or sample storage.
- Exercise good housekeeping.
- Wear close-toed shoes in lab.

Preventing Laboratory Accidents

- Lab accidents are preventable.
  - Proper chemical storage
  - Correctly transferring chemicals for use
  - Following proper laboratory techniques
  - Following laboratory safety procedures

Chemical Storage

- Storeroom should be properly ventilated, lighted, and laid out to segregate incompatible chemicals
- Clearly label and date ALL chemicals and bottles of reagents
  - Received / Opened / Expire
- Maintain Safety Data Sheet (SDS) for all chemicals used in laboratory
  - Must be maintained for 30 years after the chemicals was last on the premises

Chemical Storage

- Must have current chemical inventory list available
- Do NOT keep expired chemicals
  - There is no need to keep them as they CANNOT be used for anything.
- Store acids and bases in separate storage cabinets designed for acid and base storage
- Only compatible chemicals are stored together
  - Everything *not* stored in alphabetical order
    - e.g., sodium hydroxide and sulfuric acid
- Store heavy items on or near to the floor as possible

Chemical Storage

- Secondary containment for stored chemicals must be provided.
  - Separate polyethylene trays for acids and bases
    - Cannot both dump into same tray
- Chemicals stored at safe levels, in cabinets or on stable shelving (but not on high levels)

Chemical Storage - Flammables

- Must be stored in a flammable cabinet and/or explosion-proof storage refrigerator
- Keep stored away from sources of heat or ignition
- Do not store along path of egress or in aisles

Proper Laboratory Techniques

- Faulty technique is one of the chief causes of accidents and is one of the most difficult to correct
- Acids
  - Always rinse outside of acid bottle before opening
  - Do not lay stopper/lid on counter
  - Keep all acids tightly stoppered when not in use
  - Ensure no spills were made or remain
  - Always add acid to water. Never add water to acid.

Proper Laboratory Techniques

- Labeling
  - All chemical containers need to be properly labeled to identify their contents and associated hazards.
  - Proper labeling enables quick decision making and action during an emergency.
  - Any chemical that is not labeled must be assumed to be hazardous, and disposal costs for hazardous materials can be quite high.

Proper Laboratory Techniques

- Labeling – Best Practices
  - Use both the chemical name and formula as a double check
  - Clearly indicate the concentration
  - Coordinate the labels with the SDS
    - Include any special precautions
  - Use labels and ink that are not soluble in water
  - For reagents made on site, include the name/initials of person making reagent and date it was made in addition to the other labeling requirements.

Laboratory Practices

- Use appropriate personal protective equipment and wash your hands regularly when working with chemical reagents, especially before meals or snacks.
- Food, drink, and tobacco are not allowed in the laboratory.
- Food and drink should not be stored or prepared in laboratories or chemical storerooms.
- No pipetting by mouth!

Laboratory Practices

- Always wear appropriate attire in the lab:
  - No shorts
  - No sandals or opened toe shoes
  - No loose sleeves
  - Long hair should be tied back
- Wearing of contact lenses in the lab is strongly discouraged.
  - If it is unavoidable, advise your supervisor and co-workers so that this information is known in the event of a chemical splash in the eyes.

Accident Prevention - Electric Shock

- Do not use frayed or worn wires.
- Replace connections when shows signs of thinning insulation.
- Ground all apparatus using three prong plugs.
- Do not continue to run a motor after liquid has spilled on it.
  - Turn it off immediately and clean & dry the inside.
- All permanent wiring should be installed by an electrician with proper conduit or BX (armored) cable to eliminate any danger of circuit overloading.

Accident Prevention - Cuts

- Inspect glassware before use for cracks, scratches, and other defects.
- Remove defective glassware from use.
- Wear safety goggles when working in laboratory.
- Use two hands when carrying glassware.
  - Wear gloves if there is a risk of breakage, thermal hazard (hot or cold), or chemical contamination.

Accident Prevention - Burns

- Glass stays hot for quite some time after the red color disappears.
  - Use gloves and tongs to prevent burns.
- Spattering acids, caustic materials, and strong oxidizing solutions can cause chemical burns.
  - Use gloves, aprons, and goggles to protect yourself.
- Know location and operation of emergency deluge showers and eyewash stations.
  - Test permanent stations weekly.

Accident Prevention - Burns

- Wash affected area of body immediately with large quantities of water.
- Immediately flood eyes with water from eyewash station or special eyewash solution from a safety kit.
  - Remove any contact lenses to prevent chemical getting trapped between your eye and lens.

Accident Prevention – Toxic Fumes

- Use ventilated fume hood for volatile reagent preparation.
- Select hood that has adequate air displacement to prevent noxious fumes spreading throughout building.
- Ensure hood is properly vented to the outside.

Accident Prevention – Waste Disposal

- Hazardous materials should never be poured down an ordinary sink.
  - Corrosive substances can corrode away drainpipes.
    - Neutralize & pour down corrosion-resistant sinks & sewers
  - Flammable substances can volatilize, and the vapors may explode.
  - Chemicals may pass through the wastewater treatment plant and poison local bodies of water or groundwater.
- Use large quantities of water to dilute and flush the corrosive substance.

Accident Prevention – Waste Disposal

- Provide dedicated trash receptacle for broken glass.
  - Broken glass disposed of improperly can be a cut hazard for custodial staff.
- Always dispose of hazardous materials in accordance with local or state health or environmental regulations.
  - Refer to the SDS.

Accident Prevention – Fire

- Lab should be equipped with fire blanket, used to smother clothing fires.
- Small fires in a dish or beaker may be put out by covering the container.
- For larger fires, or ones that may spread rapidly, use the proper type of fire extinguisher for each class of fire.
- Fires classified on as A, B, C, or D fires based on the type of material being consumed.
  - Fire extinguishers are classified the same.

Accident Prevention – Fire

- Class A fire → Ordinary combustibles
  - e.g., wood, paper, cloth, rubber, many plastics, dried grass, hay, and stubble
  - Use foam, water, soda-acid, carbon dioxide gas, or almost any type of extinguisher
- Class B fire → Flammable and combustible liquids
  - e.g., gasoline, oil, tar, oil-based paint, lacquer, and solvents
  - Use foam, carbon dioxide, or dry chemical extinguishers.

Accident Prevention – Fire

- Class C fire → Energized electrical equipment
  - e.g. starters, breakers, and motors
  - Use carbon dioxide or dry chemical extinguishers
- Class D fire → Combustible metals
  - e.g., magnesium, sodium, zinc, potassium
  - Use fine dry soda ash, sand, or graphite

Accident Prevention – Fire

- Multipurpose extinguishers are also available.
  - An ABC CO_2 extinguisher will handle most laboratory fires.
- When using CO_2 extinguishers, remember CO_2 can displace oxygen, so take appropriate measures.
- There is no single type of fire extinguisher that is effective for all fires, so it is important to know the class of fire you are trying to control.
- Training must be provided in the use of the different types of extinguishers.

Chemical Fume Hoods (29 CFR 1910.1450)

- A fume hood is a ventilated enclosure in which gases, vapors and fumes are contained.
  - An exhaust fan situated on the top of the building pulls contaminated air through exhausts them to the atmosphere.
- This ventilation is often the best engineering control available and is the primary controlling device to keep chemical exposure below exposure limits.

Chemical Fume Hood Components

- Airfoil:
  - Located at the bottom and sides of the fume hood entrance the airfoil reduces turbulence in the air entering the hood near the edges.
- Air baffle and adjustable slots:
  - Used to ensure a laminar flow through the hood and to decrease turbulence.
  - Laminar -a type of flow pattern in which all the particles are flowing in parallel lines, opposed to turbulent flow, where the particles flow in random and chaotic directions

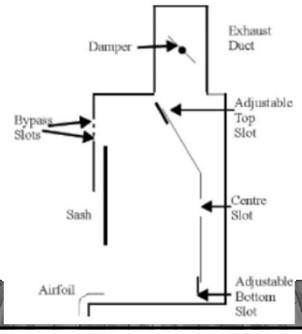
Chemical Fume Hood Components

- Sliding sash:
  - Allows full access to the fume hood when setting up an experiment and partial or full closing while running the experiment to ensure proper evacuation of hazardous products.
- Exhaust duct and damper:
  - Used to set the face velocity of the fume hood. This is normally set between 80 and 120 ft/min with the sash set at normal working height.
- Construction:
  - Material used in construction should be non-porous and impervious to materials used or produced.

Chemical Fume Hood Components

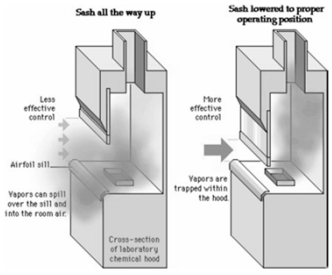
- Bypass slots:
  - Bypass slots are sequentially uncovered as the sliding sash is closed, allowing more air to enter through the bypass slots and less through the sash opening. This keeps the face velocity relatively constant.
  - If there are no bypass slots the velocity through the fume hood opening would increase as the sash is closed to a point where turbulence would force hazardous materials out of the fume hood and into the laboratory. Flow rates could also increase to the point at which experiments are physically disrupted by the air flow.

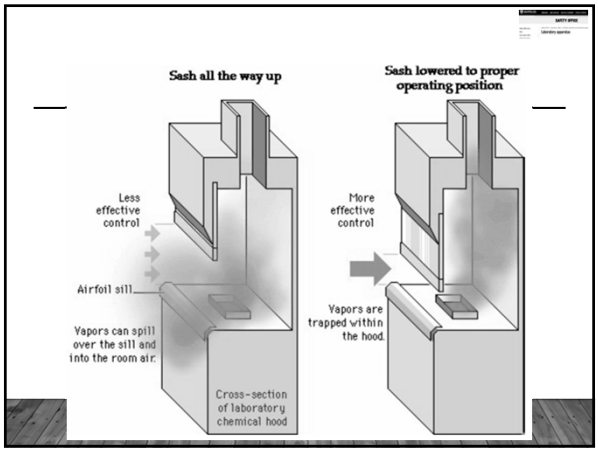
Chemical Fume Hood Components



Chemical Fume Hood

- The fume hood sash should be opened only as far as necessary to access your work.
- Always keep fume hood sash closed when the hood is not being used.





Chemical Fume Hoods (29 CFR 1910.1450)

- Before using a fume hood:
  - Make sure you understand how the hood works.
  - You should be trained to use it properly.
  - Know the hazards of the chemical you are working with.
    - Refer to SDS if you are unsure.
  - Ensure that the hood is on.
  - Make sure the sash is open to the proper operating level, which is indicated by arrows on the frame.
  - Make sure the air gauge indicates that the air flow is within the required range.

Chemical Fume Hoods (29 CFR 1910.1450)

- When using a fume hood:
  - Never allow your head to enter the plane of the hood opening.
    - For vertical sashes, keep the sash below your face.
    - For horizontal sliding sashes, keep the sash positioned in front of you and work around the side of the sash.
  - Use appropriate eye protection.
  - Be sure that nothing blocks the airflow through the baffles or through the baffle exhaust slots.
  - Elevate large equipment at least 2 inches off the base of hood interior.

Chemical Fume Hoods (29 CFR 1910.1450)

- When using a fume hood:
  - Keep all materials inside the hood at least six inches from the sash opening,.
  - When not working in the hood, close the sash.
  - Do not permanently store any chemicals inside the hood.
  - Promptly report any hood that is not functioning properly to your supervisor.
    - The sash should be closed, and the hood "tagged" and taken out of service until repairs can be made.
  - Be familiar with laboratory's action plan in case of emergency.

Autoclaves/Sterilizers

- Workers should recognize the potential for burns … while handling or sorting hot sterilized items …, and use caution when removing them from autoclaves/sterilizers or from steam lines that service the autoclaves.
- Workers should use appropriate hand protection when hands are exposed to hazards such … thermal burns.
  - Example: Using oven mitts for handling hot items

Autoclaves/Sterilizers

- Establish good work practices to prevent injuries from occurring:
  - Ensure that the autoclave/sterilizer door is closed and locked before beginning the cycle.
  - Do not remove items from an autoclave/sterilizer until they have cooled.
  - Avoid handling the sharp ends of instruments.
  - Use forceps or other tools to remove sharp instruments from baskets and autoclaves.

Autoclaves

- Prevent injuries when using the autoclave by observing the following rules:
  - Wear heat resistant gloves, eye protection, closed toed shoes and a lab coat, especially when unloading the autoclave.
  - Prevent steam burns and shattered glassware by making sure that the pressure in the autoclave chamber is near zero before opening the door at the end of a cycle. Slowly crack open the autoclave door and allow the steam to escape gradually.

Autoclaves

- Prevent injuries when using the autoclave by observing the following rules:
  - Allow items to cool for 10 minutes before removing them from the autoclave.
  - Never put sealed containers in an autoclave. They can explode. Large bottles with narrow necks may also explode if filled too full of liquid.
  - Never put solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials in an autoclave.

Handling Glassware

- Examine all glassware before use.
  - Discard any broken glass apparatus in the appropriate sharps container.
- Never store damaged glassware in cupboards.
  - Damaged glassware should either be sent for repair properly or disposed in a separate labeled container for sharps disposal.
- Use gloves when sweeping up broken glass, do not use bare hands.
  - Pick up fine glass particles with wet paper toweling.

Lab Work Area

- The work bench is to be kept clean at all times, and free from chemicals and apparatus which are not required.
- Before starting an experiment, make sure you are familiar with all the procedures and the potential hazards of the starting materials and products.
  - Determine the appropriate safeguards and remedies.
  - Know the procedures for emergency shut off as well as the person and phone numbers to contact in case of emergency.
  - If anything unexpected occurs during your experiment, or if you are in any doubt, consult your supervisor immediately.

Pregnancy

- Women who are pregnant should discuss their work assignments with their supervisors to seek alternate work assignments if the potential for exposure to teratogens exist
  - Teratogens are reproductive toxins that may cause damage to the fetus
  - THM Plus method by Hach for determining Trihalomethanes deals with chloroform, a teratogen

Chemical Spills

- Small spills (generally less than 100 mL) can usually be cleaned up safely by the employees involved.
- The hazardous properties of the material must be considered when deciding whether it is a “small” spill or not, and therefore whether unassisted clean-up should be attempted.
- Employees must be trained in advance to handle cleanup of even small spills

First Aid

First Aid

- First aid is emergency care provided for injury or sudden illness before emergency medical treatment is available.
- Policies and procedures should be communicated to all employees.
  - It is advisable to put the First-Aid Program policies and procedures in writing.
- OSHA First Aid standard (29 CFR 1910.151)
  - Employers are required to have a person or persons adequately trained to render first aid for worksites that are not in near proximity to an infirmary, clinic, or hospital.

First Aid Supplies

- First aid supplies
  - Must be adequate
  - Should reflect the kinds of injuries that occur
  - Must be stored in an area where they are readily available for emergency access
- ANSI/ISEA has two classes of first aid kits:
  - Class A - designed to deal with the most common types of workplace injuries
  - Class B - designed with a broader range and quantity of supplies to deal with injures in more complex or high-risk environments

First Aid Program

- Should be designed to reflect the known and anticipated risks of the specific work environment
- Must comply with all applicable OSHA and TOSHA standards and regulations
- First-aid supplies must be available in adequate quantities and be readily accessible

First-Aid Kits

- Well-equipped first-aid chest or kit is essential.
- Should be inspected by appropriate safety officer to ensure supplies are available and in date.
- Kits should be prominently displayed throughout treatment plant and in company vehicles.
- Each utility should establish standard operating procedures (SOPs) for first-aid treatment of injured personnel.

First Aid – Eye Burns

1. Call 911 immediately.
2. Apply a steady flow of water to eyes for at least 15 minutes.
3. Do not remove burned tissue from the eyes or eyelids.
4. Do not apply medication (except as directed by a doctor).
5. Do not use compresses.

First Aid – Gross (large) Skin Burns

1. Call 911 immediately.
2. Remove contaminated clothing immediately (preferably under a shower).
3. Flush affected areas with generous amounts of water.
4. Do not attempt to neutralize without direction of a physician.

First Aid – Swallowing or Inhalation

1. Call 911 immediately.
2. Read antidote on label of any chemical swallowed. For some chemicals, vomiting should be induced, while for others, vomiting should not be induced.
   - Refer to chemical label or SDS.

Community Public Water System Design Criteria

Division of Water Resources (DWR)
TN Department of Environment & Conservation (TDEC)
2018

TN Design Criteria Part 6 Laboratory Facilities

- 6.0 GENERAL - Laboratory equipment and facilities shall be compatible with the raw water source, intended design of the treatment plant, and the complexity of the treatment process involved. Recognized laboratory procedures must be utilized. See Parts 4 and 5 for related criteria.
- 6.1 EQUIPMENT – Laboratory and analytical equipment shall be provided to conduct all daily water quality testing as specified by the Department.

TN Design Criteria Part 6 Laboratory Facilities

- 6.2 LABORATORY SPACE AND FACILITIES
  - 6.2.1 Laboratory facilities shall be located in a separate room from office/lunch activities and from the treatment units. Facilities shall be isolated by doors and not be located in the main traffic pattern.
  - 6.2.2 Sufficient bench space, adequate ventilation, adequate lighting, storage room, laboratory sink, and auxiliary facilities shall be provided.
  - 6.2.3 The bacteriological laboratory, if provided, shall have about 6-10 feet of counter space and shall be located in a separate room or area.

TN Design Criteria Part 6 Laboratory Facilities

- 6.3 SAMPLE TAPS - Sample taps shall be provided so that water samples can be obtained from each water source and from appropriate locations in each unit operation of treatment. Taps shall be consistent with sampling needs and not be of petcock type. Sample lines and pumps where applicable shall be sized to minimize time lag between point of sampling and point of sample collection.

Laboratory Equipment

Identification, Handling and Cleaning

Fleming Training Center
Your Partner in Clean Water

Objectives

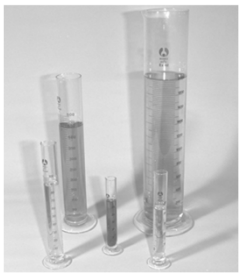
- Identify equipment commonly used in water treatment and wastewater laboratory
- Discuss accuracy and use of glassware
- Discuss how to maintain analytical equipment

Beakers

- Used for:
  - Mixing
  - Measuring approximate volumes
  - ~10% accuracy

Graduated Cylinders

- Accurate to ~1%
- Measures liquid volumes more accurately than beakers, but still not the most accurate
- Measure quicker



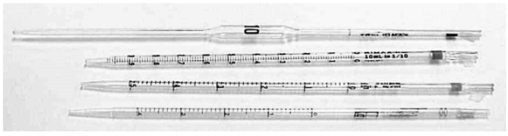
Volumetric Flasks

- Most accurate way to measure volume
- Disadvantage:
  - Only can measure one volume
  - Not used for storing or heating solutions

What Are Pipets?

- Pipets are glass or plastic tubes, usually open at both ends,which are used to transfer specific amounts of liquid from one container to another
- They are usually used for volumes between 1 and 100 milliliters

Types of Pipets



- Volumetric
- Measuring
  - Mohr
  - Serological

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Volumetric Pipets

- Used to deliver a single specific volume of liquid, usually between 1 and 100 mL
- Shaped like rolling pins with a large belly, one blunt end, the neck, and one tapering end, the tip

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Volumetric Pipets

- Used for accurate measurements, since it is designed to deliver only one volume and is calibrated at that volume
- Should be used when accuracy and reproducibility are crucial, because these can achieve accuracy to four significant figures

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Specifications on a Volumetric Pipet

- When emptying a volumetric pipet, the liquid is allowed to drain out
  - It is NOT forced out
- After it is emptied, the small amount of liquid which remains in the tip should not be blown out
- Volumetric pipets are NOT blow-out pipets

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Measuring Pipets

- They are straight glass or plastic tubes with one tapering end
- Calibrated into small divisions so that various amounts of liquid can be measured with the same pipet
- Usually used to measure any amount between 0.1mL and 25.0mL
- They are not as accurate due to the fact that any imperfection in their internal diameter will have a greater effect on the volume delivered

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Mohr and Serological Pipets

- Measuring pipets are divided into:
  - Mohr Pipets

    - Graduations on these always end before the tip
  - Serological Pipets

    - Graduation marks continue to the tip

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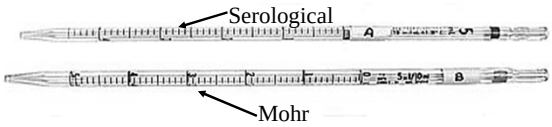
Examine pipets A and B.
Which is the serological and which is the Mohr?



Specifications on a Measuring Pipet

- Maximum volume of liquid that can be transferred
- Size of the divisions on the pipet
- Temperature at which calibrations were made
- If the pipet is a “to deliver”(TD) or “to contain”(TC) pipet.

5 mL in 1/10 mL
TD 20°C

- Specifications on a pipet as shown above indicate that the pipet is calibrated in 1/10 mL divisions and will deliver up to 5.0 mL within published tolerance levels at 20°C

1 mL in 1/100 mL
TD 20°C

- These specifications indicate that the pipet is calibrated in 1/100 mL divisions and it will deliver up to 1.00 mL within published tolerance levels at 20°C

Handling and Disposing of Pipets

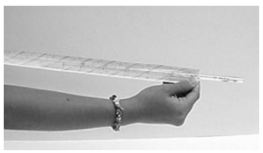
- Chipped and cracked pipets should be replaced as they are unsafe and may affect the accuracy of measurements
- NEVER mouth pipet
- Hold the pipet by the upper third of the tube and keep the tip from touching anything

Handling and Disposing of Pipets

- Dispose dirty pipets by placing in soapy water solution in a tray or pipet washer
- Place disposable pipets in a cardboard holder
- Do not leave pipets on counters or sinks

Handling Sterile Pipets

- When using sterile pipets, be sure to use proper sanitary techniques
- If you have a sterile package of disposable pipets, tear only a small corner of the package open and push one pipet out of this opening, then immediately close the package to prevent contamination



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Handling Sterile Pipets

- If you are using sterile pipets in a pipet canister, place the canister on its side, slide off the cover, pull out one pipet and replace the cover immediately

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Transferring a Precise Volume of Liquid

- A pipet bulb is used to draw liquid up into the pipet
- There are many types of pipet bulbs

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Transferring a Precise Volume of Liquid

- You should observe the meniscus at eye-level
- Touch the tip of the pipet to the inside of the container when the meniscus is at the desired level

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Transferring a Precise Volume of Liquid

- Squeeze bulb and touch it to the mouth of the pipet
- Place other end of the pipet in liquid to be transferred and slowly release pressure on bulb
- Draw liquid up past desired level, quickly replacing bulb with index finger

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Transferring a Precise Volume of Liquid

- Let liquid drain until bottom of meniscus lines up with desired level on pipet
- Touch tip of pipet to inside of beaker to remove any adhering drops
- Transfer liquid to second beaker and touch tip to inside of beaker and let liquid drain out of pipet

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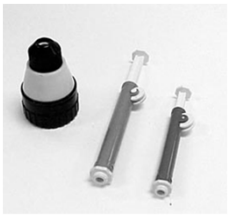
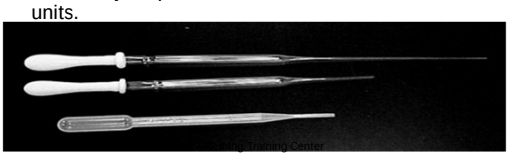
Other Pipet Bulbs

Other pipet bulbs that are often used include the Vadosa pipet filler, seen on the left, and the pipet Pumper, on the right

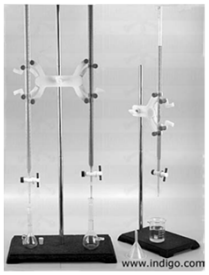


Other Pipet Types

- Transfer of uncalibrated volumes up to 2.5 mL can be accomplished using glass “transfer” or “Pasteur” pipets shown below. These may be sterilized before use.
- Roughly calibrated volumes of 1 and 2 mL can be transferred with the one piece plastic transfer pipets which may be purchased as sterile or non-sterile units.



Burettes and Titrations



- Burettes
  - Used for titrations
  - Treat like a Mohr pipet, do not let liquid completely drain out
  - Also, make sure to remove air bubble in tip before titrating

Flasks

- Distilling Flask

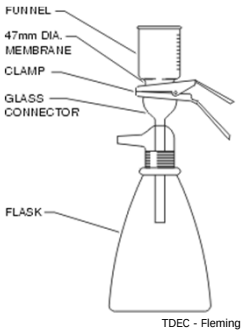
- Florence (Flat Bottom) Flask

Flasks

- Erlenmeyer Flasks

- Filter Flask

Filter Apparatus



- Vacuum Pump

Bottles

- Dilution Bottle
- Sample Bottles

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Bottles

- Reagent Bottle
- Weighing Bottles

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Funnels

- Separatory
- Buchner
- General

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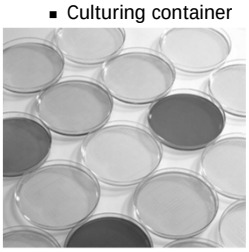
- Petri Dish
  - Culturing container
- Desiccators
  - Dust and moisture free



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- Evaporating Dish
- Crucible

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Centrifuge

- Used to separate materials of different density

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Autoclave

- Pressure cooker used to sterilize glassware, bottles, membrane filter equip, culture media and contaminated material to be discarded.
- Standard temperature is set at121°C and 15 PSI

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Refrigerators

- Sample storage should maintain between 1-5 °C
- Never store samples and chemicals together

Explosion proof

Walk-in cooler

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Incubators

- Artificially heated container used for growing bacteria cultures
- Dry-Heat types hold temperatures to ± 0.5°C
- For E. coli and Total coliform = 35 ± 0.5°C
- Water Bath for fecals = 44.5 ± 0.2°C

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Incubators

- For BOD incubation at 20 ± 1 °C
- Do not store chemical solutions and samples in same refrigerator

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UV Sterilizer

- Use in Bac'T Lab to sterilize test equipment

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Drying Oven

Used more often in wastewater labs

For some solids testing set oven at 103-105°C

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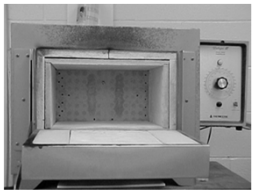
Muffle Furnace



- High temp oven used to ignite or burn solids.
- Usually operate at temps of 550°C.
- More often used in Wastewater lab work.

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Fume Hood

- Can prevent serious accidents
- Use whenever heat is used in a test procedure
- Fumes vented out of lab
- Use when harmful smoke, gas, vapors, splashes or fumes are possible

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Water Still

- Produces distilled water for lab tests and rinsing washed glassware
- Removes dissolved minerals, organic and inorganic nonvolatile compounds
- Does not sterilize

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Heating and Stirring Samples

- Combo Heat/Stir Plate
  - Can be used to stir or heat and stir samples
  - Safer than heating with an open flame

- Gas Burner
  - Uses natural gas

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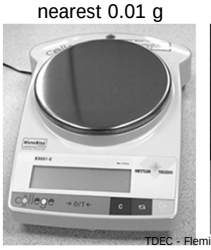
Balances

- Top Loading
  - Weighs to the nearest 0.01 g
- Analytical
  - Precise to 0.0001 g



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pH Meter

- Use buffer solutions to calibrate
- Store electrodes properly
- Calibrate daily
- Maintain records on daily calibrations

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Spectrophotometer

- HACH DR 6000
  - Factory pre-set programs for lab chemical analysis
- Very versatile

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Colorimeters

- Determine the concentration of many chemicals
- Most commonly used is chlorine type colorimeter
- Portable and battery powered

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Amperometric Titrator

- Chlorine analysis
- Accurate and unaffected by sample color or turbidity
- Takes greater skill to use than DPD method with colorimetric devices

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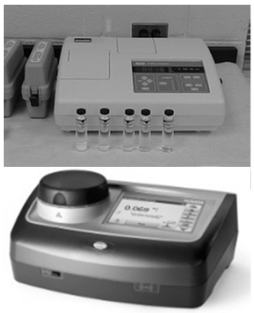
Turbidimeter



- Desk top and continuous on-line monitoring
- Position away from direct sunlight and have extra light bulb on hand
- Ensure sample bottles maintained; no scratches; acid clean if necessary

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Chemical Storage

- Do not store volatile chemicals together
- Have separate storage cabinets for acids and bases/caustics

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Flammable Cabinet

- Flammable chemicals should be kept in a flammable cabinet.

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Safety Equipment

PPE (Personal Protective Equipment):

Goggles

Gloves

Aprons

Wear safety clothing.

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Eye Wash and Shower

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Cleaning Glassware

- Just because it looks clean does not mean residues are not left behind
- Results need to be accurate to use data for process control and/or reporting to the State
- Detergents, such as Alconox, may be sufficient
  - Should be phosphate-free

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Cleaning Glassware

- Residues of minerals and other substances can build up on glassware, causing erroneous test results

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Steps for Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse at least three times with distilled water
- Let air dry

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Steps for Acid-Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse with 1:1 hydrochloric acid or nitric acid
  - 1:1 means equal parts distilled water and acid
- Rinse well with distilled water
- Let air dry

Note: always use gloves and goggles when handling acids

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Cleaning Glassware

We often overlook the importance of clean glassware in the lab. We think if it looks clean, it must be clean. But there may be residues on the glassware that can affect our results. Since we use those results for both running the plant and reporting water quality to the state, it is important that those results be as accurate as possible.

For many purposes in the water treatment lab, washing in a detergent such as Alconox is sufficient. However, some analyses and some glassware require special cleaning procedures to ensure removal of all residues. Residues of minerals and other substances can build up on glassware, causing erroneous test results. Always follow the recommendations for cleaning glassware and sample containers, and always use the suggested type of sample container.

The following is a partial list of special cleaning procedures for laboratory glassware used for chemical analyses:

Sample containers:

When collecting samples for metals analyses, special cleaning of the containers is necessary to prevent residues from affecting results. Clean the sample bottles by thoroughly washing with laboratory soap (preferably phosphate-free), followed by an acid wash and multiple rinses with distilled or deionized water. Do not use glass sample bottles for metals analysis.

Sample cells and cuvets:

Wash thoroughly using laboratory soap (preferably phosphate-free), followed by an acid wash and multiple rinses with distilled or deionized water. Allow to air dry or wipe with a Kim-wipe, don't use paper towels.

Flasks, beakers, etc used for metals analysis:

Wash thoroughly using laboratory soap (preferably phosphate-free), followed by an acid wash and multiple rinses with distilled or deionized water.

Pipets:

Soak overnight in a solution of Alconox. Rinse thoroughly using a pipet washer.

Procedure for Acid Washing Glassware

If acid washing is required, follow these steps:

- Clean the glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse with 1:1 hydrochloric acid solution or 1:1 nitric acid solution
- Rinse well with distilled water
- Air dry
- ❖ Note: always use gloves and safety goggles when handling acids

Quality Assurance/Quality Control (QA/QC)

1

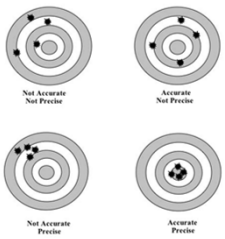
Terms

- A QA/QC program consists of procedures that ensure the precision and accuracy of tests performed on a daily basis.
- Precision – repeatability; being able to get the same results time after time
  - Shooting at a target and hitting the same spot repeatedly
- Accuracy – closeness of test results to the correct (known) value
  - Shooting at a target and hitting bull's eye

2

Precision and Accuracy

- Accuracy
  - Correctness
  - Checked by using a different method
  - Poor accuracy results from procedural or equipment flaws
- Precision
  - Reproducibility
  - Check by repeating measurements
  - Poor precision results from poor technique



3

Three Phases of QA/QC

- Record keeping
- Quality Assurance
  - Documenting that equipment is regularly calibrated and temperatures are correct
- Quality Control
  - Perform tests to demonstrate precision and accuracy

4

Record Keeping

- Maintain a complete and accurate list of exact locations of all sampling sites
- Maintain a complete and accurate list of all test procedures used
  - Record method numbers on bench sheets
- Write in pen
- Initial your entries
- Use a notebook that has numbered pages

5

Laboratory Conditions

- Lab equipment should be in good working condition
  - Calibrated
  - Checked against standards
- Lab quality distilled water and fresh, pure reagents must be on hand
- Lab temperature should be 68°F

6

Laboratory Conditions

- Glassware
  - Keep it clean
- pH meter
  - Calibrated daily w/ fresh buffers
- Spectrophotometer
- Incubator
  - Keep records of temperatures
- Balances
  - Checked at least annually for accuracy
  - Use ASTM Class 1 weights to check calibration

7

Laboratory Conditions

- Whenever repairs or maintenance are done on lab equipment, record those activities in a logbook

8

Reagents

- Use only fresh ACS (American Chemical Society) Reagent Grade chemicals
- Check for expiration dates
- Mark chemicals with date arrived in lab, date opened and expiration date
- When solutions are mixed using dry reagents, record this in a reagent log
  - If standardization is required, include this information in the reagent log as well

9

Distilled & Deionized Water

- The quality of distilled or deionized water can greatly influence the quality of the test.
- There should be no traces of chlorine, ammonia and total suspended solids.
- TDS should be less than 1 mg/L.
- Distilled is better than deionized.
- Ultrapure is better than distilled.

10

Quality Control Tests

- Duplicates
- Blanks
- Lab Standards
- Unknown Lab Standards
- Spikes

- For frequency of each QC test, refer to 40CFR141.

11

Duplicates

- Simplest form of QC test.
- Run two tests on one sample.
  - This shows how precise the analyst's procedure is.
  - Sample results should yield very close results (goal is to have no difference).
- Duplicate samples should be analyzed at a minimum of 5% of samples run in an analytical batch.
  - i.e., one sample duplicate for every 20 samples
  - TSS duplicates should be run more often.

12

Duplicates

- Common sources of error:
  - Sample size (should be the same size)
  - Insufficient mixing
  - Dirty glassware
  - Calculation errors
  - Titration (misreading the burette)
  - Weighing
  - Calibration
  - Reagent water
  - Reagents

13

Duplicates

- Relative Difference
  - A quantitative indicator of quality assurance and quality control for repeated measurements where the outcomes are expected to be the same.
  - Compares one value to another (ratio) and helps to determine the percentage increase or decrease from one value to another.
  - To find the relative difference between two values, divide the difference by the original value:

$$\text{relative difference}, \% = \frac{\text{difference in values}}{\text{original value}} \times 100, \%$$

14

Duplicates

- Relative Percent Difference (RPD)
  - A comparative statistic used to calculate precision or random error.
  - Defined as the absolute value of the difference between two results divided by the mean of the two results.

$$\text{RPD}, \% = \frac{|\text{Reference value} - \text{new value}|}{\text{arithemetic mean}} \times 100, \%$$

15

Duplicates

- Range
  - Take absolute value of the relative difference between the two test results. (will never be negative)

$$\text{Range} = |\text{Original Sample Value} - \text{Duplicate Sample Value}|$$

- Average
  - Sum of measurements divided by # of measurements

$$\text{Average} = \frac{\text{Sum of measurements}}{2}$$

- Relative Percent Difference (RPD)
  - Divide the range by the average, multiply by 100.

$$\text{RPD}, \% = \frac{\text{Range}}{\text{Average}} \times 100, \%$$

16

Example

A raw water sample was tested for TDS concentration. The sample read 16.3 mg/L, while the duplicate sample read 19.5 mg/L. Calculate the RPD (%).

1. Calculate the Range.

$$\text{Range} = |\ 16.3\ \text{mg/L} - 19.5\,\text{mg/L}\ | = 3.2\ \text{mg/L}$$

2. Calculate the Average.

$$\text{Average} = \frac{(16.3\,\text{mg/L} + 19.5\,\text{mg/L})}{2} = \frac{35.8\,\text{mg/L}}{2} = 17.9\,\text{mg/L}$$

3. Calculate RPD.

$$\text{RPD}, \% = \frac{3.2\ \text{mg/L}}{17.9\ \text{mg/L}} \times 100(\%) = 17.9\ \%$$

Is this an acceptable RPD?

17

Blanks

- Sample of deionized or distilled water that is run through the test method same as a sample.
  - Treat blank just as you do the sample.
    - Take through all procedures
    - Add all reagents or incubate along with other samples
- Target value for blanks is zero.
- Positive blanks shows a problem:
  - Bad reagents
  - Bad technique
  - Unclean glassware
  - Bad distilled water

18

Blanks

- Bacteriological Testing
  - A blank should never be positive.
  - Membrane Filtration Method
    - If the pre-sample blank has colony growth, the equipment was not properly sterilized.
    - If the post sample blank has colony growth, the equipment was not cleaned well enough between samples.

19

Laboratory Standards

- Determine accuracy.
- If the test value agrees with the true (given) value, the test has been performed accurately.
- Can be mixed onsite or purchased from a supplier.
  - Purchased standards should be the preference because this can reduce the possibility of having mixing errors; they also come with a certificate of analysis
- Perform along with dups., one every 10 samples

20

Performance Evaluation (PE) Samples

- Samples that evaluate the overall bias of the analytical laboratory and detect any error in the analytical method used.
- Typically prepared by a third party, using a quantity of the analyte(s) of interest which is known to the preparer but unknown to the laboratory, and always undergo certification analysis.
- Laboratory procedural error is evaluated by the percentage of analyte identified in the PE sample.
- Equivalent to matrix spikes prepared by a third party that undergo certification analysis and can be non-blind, single-blind, or double-blind.

21

Unknown Laboratory Samples

- EPA quality control unknowns
- Commercially available
- Gives confidence to analyst
- Can show deficiencies in the testing procedure

22

Spikes

- Determines accuracy
- A known amount of standard is added to a sample
- The results should equal the sample value plus the added known amount
- Goal is to have 100% recovery of spike and sample
- Example:
  - A sample was read and had a chlorine concentration of 1.3 mg/L. If the sample is spiked with 0.5 mg/L, the new measurement should yield 1.8 mg/L.
    - < 1.8 = <100% recovery
    - > 1.8 = > 100% recovery

23

Split Samples

- Some labs split samples with other labs to check the accuracy of the testing procedure
- If you are concerned that your contracted lab is getting wrong values, send in a known standard as a sample
  - This does double you cost, but you can see how close they are to the known value
  - Don't tell the contracted lab that the second sample is a known

27

40CFR136

Method Update Rule
For Backwash Lagoons that have Discharge Permits
Fleming Training Center

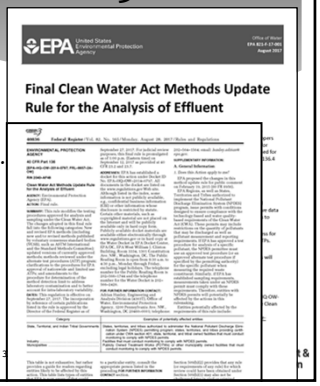
You Have Heard it All Before

* More Rules
* More Testing
* More Paperwork
* More Cost

* But everything we do is regulated.

2017 Update of 136

* Standard Methods approved by date not Edition
* Section 136.7 Quality Assurance and Quality Control.
* Appendix B to Part 136 – Definition and Procedure for the Determination of the Method Detection Limit – Revision 2

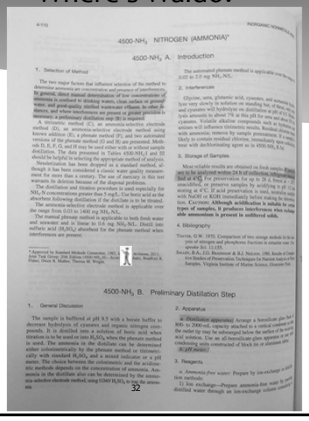

EPA United States Environmental Protection Agency

Final Clean Water Act Methods Update Rule for the Analysis of Effluent

"Standard Methods" Changes

* Approved methods are not listed by the Edition of Standard Methods, but by the year the method was approved…

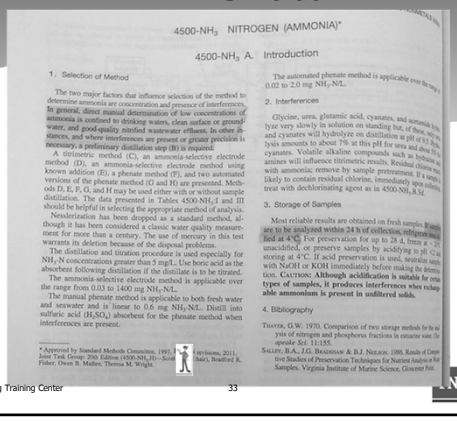
Where's Waldo?


4500-NH_3 NITROGEN (AMMONIA)*

4500-NH_3 A. Introduction

4500-NH_3 B. Preliminary Distillation Step

THERE'S Waldo!


4500-NH_3 NITROGEN (AMMONIA)*

4500-NH_3 A. Introduction

40 CFR 136 as of 8/28/17 Table 1B

Parameter	Methodology	EPA	Standard Methods	ASTM	USGS/AOAC/ Other
Chlorine-Total Residual, mg/L	Amperometric Direct	.....	4500-Cl D-2011	D1253-08	
	Amperometric Direct (Low Level)	.....	4500-Cl E-2011		
	Iodometric Direct	.....	4500-Cl B-2011		
	Back Titration Ether End-Point[15]	.....	4500-Cl C-2000		
	DPD-FAS	.....	4500-Cl F-2000		
	Spectrophotometric, DPD	.....	**4500-Cl G-2000**		
	Electrode	.....	.....	.....	See Footnote [16] & I

40 CFR 136 as of 8/28/17 Table 1B

Parameter	Methodology	EPA	Standard Methods	ASTM	USGS/AOAC/ Other
Hydrogen Ion (pH), pH units	**Electronic Measurement**	.....	**4500---H^+ B-2011**	D1293-99 (A or B)	973.41, [3] I-1586-85.[2]
	Automated Electrode	150.2 (Dec. 1982)[1]	.....	.....	See Footnote, [21] I-2587-85[2]

40 CFR 136 as of 8/28/17 Table 1B

Parameter	Methodology	EPA	Standard Methods	ASTM	USGS/AOAC /Other
Residue – Non-filterable (TSS), mg/L	**Gravimetric, 103-105° post washing of residue**	.....	**2540 D-2011**	D5907-03	I-3765-85.[2]
Residue – Settleable, mg/L	**Volumetric, (Imhoff cone) or gravimetric**	.....	**2540 F-2011**		
Temperature, °C	**Thermometric**	.....	**2550 B-2010**	.....	See Footnote.[32]

40 CFR 136 as of 8/28/17 Table 1B

Parameter	Methodology	EPA	Standard Methods	ASTM	USGS/AOAC/ Other
Aluminum – Total, mg/L	**Digestion, followed by any of the following**				
	• AA direct aspiration	.....	3111 D-2011 or 3111 E-2011	.....	I3051-85
	• AA furnace	.....	3113 B-2010		
	• STGFAA	200.9 Rev 2.2 (1994)			
	• ICP/AES	200.5 Rev 4.2 (2003), 200.7 Rev 4.4 (1994)	3120 B-2011	D1976-07	I-4471-97
	• ICP/MS	200.8 Rev 5.4 (1994)	3125 B-2011	D5673-05	993.14, I-4471-97
	• Direct Current Plasma (DCP)	.....	.....	D4190-08	
	• Colorimetric (Eriochrome cyanine R)	.....	3500-Al B-2011		& I

40 CFR 136 as of 8/28/17 Table 1B

Parameter	Methodology	EPA	Standard Methods	ASTM	USGS/AOAC/ Other
Iron – Total, mg/L	**Digestion, followed by any of the following**				
	• AA direct aspiration	.....	3111 B-2011 or 3111 C-2011	D1068-05	974.27, I-3381-85
	• AA furnace	.....	3113 B-2011	D1068-05	
	• STGFAA	200.9 Rev 2.2 (1994)			
	• ICP/AES	200.5 Rev 4.2 (2003), 200.7 Rev 4.4 (1994)	3120 B-2011	D1976-07	I-4471-97
	• ICP/MS	200.8 Rev 5.4 (1994)	3125 B-2011	D5673-05	993.14
	• Direct Current Plasma (DCP)	.....	.....	D4190-08	
	• Colorimetric (Phenanthroline)	.....	3500-Fe-2011	D1068-05	& I

Three QA Options

* A. …follow equivalent EPA procedures
* B. Refer to QA/QC in consensus organization compendium. (Follow Standard Methods) didn't we have that on the previous slide?
* **C. Follow the 12 Steps where applicable.**

12 Quality Control Elements

1. DOC – demonstration of capability
2. MDL – method detection limit
3. LRB/MB – method blank
4. LFB – laboratory fortified blank (standard)
5. LFM/LFMD – laboratory fortified matrix/duplicate (spike)
6. Internal standards, surrogate standards or tracer – ***only applies to organic analysis and radiochemistry***
7. Calibration- initial and continuing
8. Control charts or other trend analysis
9. Corrective action – root cause analysis
10. QC acceptance criteria
11. Definition of a batch (preparation and analytical)
12. Minimum frequency for conducting all QC elements
13. Unwritten 13th Step – SOP – Standard Operating Procedures need to be written and followed for all lab sampling and analyses

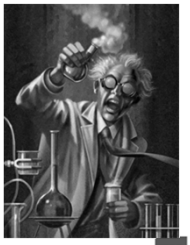
Not all of these items apply to all tests, there are many exceptions!

Can you defend what you do?

- How do you interpret your Permit language or the Rule?
- Can you defend that interpretation, will a judge or jury support you?
- What do Regulators say and what is written?
  - Is it clear?
  - Don't be afraid to ask Why?
  - Don't be afraid to ask for directives in writing.

Applicable Tests for Drinking Water

- Total Residual Chlorine
- pH
- TSS
- Settleable Solids
- Aluminum



What You Are Already Doing

- Most Labs are doing lots of QA/QC stuff
- Write down what you do….SOP
- Summarize QC Data
  - Table Form
  - Average, Max, Min.
  - Control Charts

Demonstration of Capability

- DOC
- Standard Methods 1020.B.1
  - As a minimum, include a reagent blank and at least 4 LFBs at a concentration between 10 times the MDL and the midpoint of a calibration curve.
- Standard Methods 2020B.1.a, 4020B.1.a. & 5020.B.1.a
  - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
  - LFB initial recovery limits = Mean ± (5.84xStandard Deviation)

Demonstration of Capability

- What tests does this apply to?
  - Chlorine, pH, TSS
  - Analyst needs to make up this standard, cannot be bought premade
  - Example: for chlorine, the analyst needs to make up 0.5 mg/L, not purchase pre-made 0.5 mg/L
    - Analyst can make 1 L of 0.5 mg/L by diluting down from 100 mg/L or 1000 mg/L and then pour up 4-100 mL aliquots

Demonstration of Capability

* How often?
  * Once for each analyst.
    * Recommended yearly for backup analyst who does not perform tests frequently
  * Each analyst should have a file kept on their training within and for the lab.
  * Something to keep along with these records is a signed form (documentation) that analyst has read and understands all appropriate SOPs and Methods.

Method Detection Limit (MDL)

* New definition of the MDL: "The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results."

What is an MDL study?

* It is a calculation that statistically gives the lowest concentration that a lab/facility can "see", that is detect as an analyte
* Not practical for many analyses
* As detector sensitivity improves, the background contamination of the lab, consumable supplies, and equipment can be more important in determining the detection limit than the sensitivity of the instrument
* **2014 Update – this is your reporting limit**

Method Detection Limit

- Estimation of the detection limit for variety of physical and chemical methods
- How often?
  - Annually – but at least every 13 months
- Ongoing data collection and MDL validation is now required quarterly

Method Detection Limit

* *The implied requirement that the calculated MDL must be no less than one-tenth the spike value in Revision 1.11 of the MDL procedure* ***has been eliminated in Revision 2, so laboratories will not have to redo the MDL procedure if the calculated MDL is lower than expected.***
* Latest update effective September 27, 2017.
  * Required to calculate the MDL using spiked samples and blank samples for multiple instruments (MDL samples are a reference matrix, such as reagent water, spiked with a known and consistent quantity of the analyte).
  * Required to check MDL values once a quarter instead of just once per year.
  * Recalculation of the MDL is required annually (at least once every thirteen months).

Method Detection Limit

* Standard Methods 1020.B.4
  * As a starting point for selecting the concentration to use when determining the MDL, us an estimate of five times the estimated true detection level.
  * Prepare and analyze at least seven portions of this solution over a 3-day period to ensure the MDL determination is more representative of routine measurements as performed in the laboratory.
  * Ideally use pooled data from several analysts rather than data from one analyst.

Method Detection Limit

- Determine Initial MDL:
  - Process a minimum of 7 spiked samples and 7 method blank samples
  - Must be prepared in at least 3 batches on 3 separate dates and analyzed on 3 separate dates (Preparation and analysis may be on the same day).
  - If multiple instruments will be assigned the same MDL, sample analyses must be distributed across all of the instruments.
    - Minimum two spiked and two blank samples per instrument, analyzed on different dates
  - Compute MDL_s – value based on spiked samples
  - Compute MDL_b – value based on method blank samples
  - Initial MDL is the greater of MDL_s or MDL_b

How MDL Studies are Performed

* Ongoing Data Collection:
  * Procedure uses method blanks, as well as spiked samples to calculate MDL
  * MDL_s: value calculated from the spiked samples
    * Min 2 spiked samples on each instrument
    * Min 8/year (2/quarter)
  * MDL_b: value calculated from the method blanks
    * No additional sample required, use your routine method blanks

How MDL Studies are Performed

* Annual Verification:
* At least once every 13 months, re-calculate MDLs and MDL_b
  * Ideally, use all method blank results from last 24 months for MDL_b calculation
* The verified MDL = the higher of the two numbers
  * Existing MDL may be left unchanged if specific criteria are met

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MDL Calculations

* Samples used to calculate MDL should be performed throughout the year, not on a single date
  * Samples analyzed every quarter, but calculation performed only once a year
* Lab has the option to pool data from multiple instruments to calculate one MDL that represents multiple instruments

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Method Detection Limit

* What tests does this apply to?
  * Chlorine
* How often?
  * Annually

TDEC - Fleming Training Center 56

Method Detection Limit (MDL)

* Where do I find the MDL Calculator?

1. Go to Fleming Training Center's website
   * https://www.tn.gov/environment/program-areas/wr-water-resources/fleming-training-center.html
2. On left side, click on "Course Books and Reference Material"
3. In the drop-down menu, click on "Wastewater Information"
4. Click on "Method Update Rule – Method Detection Level Math 2018"

Method Detection Limit (MDL)

- Some things to remember about the calculator…
  - Record values in multiple places so you don't lose them
  - You need to "save as" for each parameter
  - Save a copy of the calculator before you change the I3 cell date (every 13 months)
  - You need an electronic or a hardcopy on file
    - Play it safe – have both

Good MDL Values for Cl_2

Date	Analyst	Number	True Value	Value Read	% Recovery (50-150%)
1/28/2013	SEP	1	0.05	0.09	180.00
1/28/2013	SEP	2	0.05	0.07	140.00
1/28/2013	SEP	3	0.05	0.07	140.00
1/30/2013	SEP	4	0.05	0.08	160.00
1/30/2013	SEP	5	0.05	0.08	160.00
2/1/2013	SEP	6	0.05	0.07	140.00
2/1/2013	SEP	7	0.05	0.08	160.00
			Standard Deviation	**0.007559289**	
		Relative Standard Deviation (RSD)		9.7990789	(Needs to be ≤ 20%)
			MDL	**0.0237362**	

Laboratory Reagent Blank

- LRB
- Also known as Method Blank
- Standard Methods 1020.B.5
  - A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure (distillation, incubation, etc.)
- What tests does this apply to?
  - Chlorine, TSS
- How often?
  - Depends on method QA/QC

Laboratory Fortified Blank

- LFB
- Standard Methods 1020.B.6
  - A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added
  - Sample batch = 5% basis = 1 every 20 samples **(or day of for monthly reporting purposes)**
  - Use an added concentration of at least 10 times the MDL, or less than or equal to the midpoint of the calibration curve

Laboratory Fortified Blank

- Standard Methods 2020.B.2.e – TSS
  - Using stock solutions, prepare fortified concentrations so they are within the calibration curve
- Standard Methods 4020.B.2.e – Chlorine
  - Calculate percent recovery, plot control charts and determine control limits
- What tests does this apply to?
  - Chlorine, TSS

Laboratory Fortified Blank

- How often?
- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
  - If a permit stated that 3 analyses per week, we would allow for a LFB to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would suggest twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

Duplicate

- Dup
- Not a part of the 12 Steps of QA, an addition from the State of TN
- Standard Methods 1020.B.8
  - As a minimum, include one duplicate sample with each sample set or on a 5% basis
- Standard Methods 1020.B.12
  - Calculate the RPD (relative percent difference)
  - Equal to or less than 20% RPD

Duplicate – Cl_2 and pH

- Standard Methods 4020.B.2.f
  - Randomly select routine samples to be analyzed twice
  - Process duplicate sample independently through the entire sample preparation and analysis
  - Include at least one duplicate for each matrix type daily or with each batch of 20

Duplicate

- What tests does this apply to?
  - Chlorine, pH, TSS and Settleable Solids
- How often?
- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria: (10% would be once every 10 samples for TSS)
  - If a permit stated that 3 analyses per week, we would allow for a dup to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would suggest twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

Initial Calibration Verification & Continuing Calibration Verification

- ICV
  - Standard Methods 1020.B.11.b
    - Perform initial calibration using at least three concentrations of standards for linear curves
  - Calibrate pH meter or verify scale, thermometer and colorimeter/spectrophotometer

Initial Calibration Verification & Continuing Calibration Verification

- CCV
  - Standard Methods 1020.B.11.c
    - Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
    - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
  - Verify the calibration (especially if preset by manufacturer) at beginning of day, after every 10 readings and at the end of the batch
  - **Daily (or day of for monthly reporting purposes)**

Corrective Action

- Standard Methods 1020 B.15
  - QC data that are outside the acceptance limits or exhibit a trend are evidence of unacceptable error in the analytical process.
  - Take corrective action promptly to determine and eliminate the source of error.
  - Do not report data until the cause of the problem is identified and either corrected or qualified (see Table 1020:II)

Corrective Action

* The corrective action plan needs to be in your SOP for each method on what to do if your QC tests fail or are out of range
* If you have a "boo boo", write down how you fixed it
* Any issues should be recorded and a sentence on how it can be prevented, if possible, in the future
* Common problems and their corrections should be covered in your Standard Operating Procedures (SOP)
  * If you see things frequently, you can give them qualifiers that are noted in your SOP

QC Acceptance

* Have in SOP for each method the acceptance ranges for standards, duplicates, etc. and make sure they match the method requirements.
* If not mentioned in method, these are the accepted criteria for QC:
  * Blank < reporting limit
  * LFB ± 15%
  * ICV/CCV ± 10%
  * RPD ± 20%

Batch Size

* Each "Batch" could be daily (day of testing), every 10 samples or every 20 samples.
* Check method
* If you sample only once a month, need to run QC each time.

QC Frequency

* Usually lumped in with the definition of a "batch" and should be in the SOP of some kind

Standard Operating Procedure

* Here's that "13th Step", your SOP
* All procedures must be documented in some type of SOP
* It can be very simple but must provide the information necessary for someone who is not familiar with the test to perform it
  * Step by step instructions on how and where to collect the samples and then how to run the test.
* It must include the QC Acceptance Criteria, the definition of a "Batch" and the minimum frequency of QC checks

Standard Operating Procedure

* Common documents in an SOP:
  * Copy of method from manufacturer on how to calibrate instrument, run samples, etc.
  * QA/QC information from TDEC
  * Step-by-step instruction for you plant on the 12 Steps that apply to that test.
  * Where you grab your samples

12 Steps of Lab Quality Assurance

Parameter	Method	DOC	MDL	Method Blank	LFB	LFM / LFMD	Dup	ICV / CCV	Control Charts	Corrective Action	QC Acceptance	Batch Size	*QC Frequency
Ammonia	SM4500-NH3 D - 2011	X	X	X	X	X		X, Calibrate meter daily	X	X	X	20	Depends on Permit
BOD_5 / $CBOD_5$	SM5210 B - 2011	X		X	X		X	X, Calibrate meter daily	X	X	X	20	Depends on Permit
Chlorine, TR	SM4500-Cl G - 2011	X	X	X	X		X	X, verify meter daily w Secondary Gel Standards	X	X	X	20	Depends on Permit
pH	SM4500-H+ B - 2011	X					X	X, Calibrate meter daily		X	X	20	Depends on Permit
Oxygen, dissolved	SM4500-O G - 2011	X					X	X, Calibrate meter daily & verify with air-saturated water		X	X	20	Depends on Permit
	Hach Method 10360 Luminescence Oct. 2011	X					X	X, Calibrate meter daily & verify with air-saturated water		X	X	20	Depends on Permit
Phosphorus, total	SM4500-P B and E - 2011	X	X	X	X	X		X, verify meter	X	X	X	20	Depends on Permit
TSS	SM2540 D - 2011	X	X*	X	X		10%	X, verify scale daily		X	X	20/10	Depends on Permit
Sett. Solids	SM2540 F - 2011						X			X		20	Depends on Permit
Temperature	SM2550 B - 2010							X, verify against NIST thermometer		X			Annually

DOC – Demonstration of Capability

- Each analyst should have a file kept from where they have calibrated and analyzed 4 standards to demonstrate they can accurately run this test
- Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods
- Recommend backup analyst do this once a year

MDL – Method Detection Limit (Effective September 27, 2018)

- Initial MDL, run at least 7 (spiked) samples at low levels and 7 method blanks
- On-going, each quarter, analyze a minimum of 2 (spiked) samples
- On-going method blank population should include all routine method blanks analyzed with each batch during the course of sample analysis
- For ongoing annual verification - need at least 2 spikes and at least 2 method blanks each quarter for the past 24 months (unless no analysis performed)
- Annual (every 13 months) verification required using 7 (spiked) samples at low levels and 7 method blanks
- More detailed discussion can be found in the MDL Examples and EPA Guidance document, as well as 40 CFR 136 Appendix B.
- * For TSS, only the method blanks are required to determine the MDL. (*MDL_b only.*)

Method Blank – aka Laboratory Reagent Blank (LRB)

- Analyze distilled/deionized water as a sample

LFB – Laboratory Fortified Blank

- Analyze a known standard

LFM/LFMD – Laboratory Fortified Matrix/Laboratory Fortified Matrix Duplicate

- Analyze a sample with a known amount of standard added (spike)

Dup – Duplicate
- Analyze the same sample twice

ICV/CCV – Initial Calibration/Continuing Calibration Verification
- Calibrate meter (DO, pH, ISE) or verify balance, thermometer and colorimeter/spectrophotometer
- Verify the calibration (especially if preset by manufacturer) at beginning of day and/or after every 10 readings, whichever comes first.

Control Charts
- Create and maintain control charts if you have 20-30 data points within 90 days.
- If you do not meet the above criteria, follow QC Acceptance Criteria below.

Corrective Action
- Have corrective action plan in SOP for each method on what to do if QC tests fail or are out of range.
- For example, if standards fail, re-calibrate and run test again.

QC Acceptance
- Have in SOP for each method the acceptance ranges for standards, duplicates, spikes, etc. and make sure they match the method requirements.

Batch Size
- Each batch could be daily, every 10 samples or every 20 samples. Check method.

*QC Frequency (depends on permit) – at least once a month
- For samples that need to be analyzed on a 5% basis or once for every 20 samples, follow these criteria:
  - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would allow twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
  - **Please note that influent and effluent samples count as two separate samples. For example, if you are supposed to run 3 BODs a week, that should be counted as running 6 samples for that week.**
- For samples that need to be analyzed on a 10% basis or once for 10 samples, follow these criteria:
  - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least twice per month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would allow a duplicate to be analyzed once per week.
    - Pick a date and be consistent, every Monday or Wednesday. Mark your calendar!!
  - **Please note that influent and effluent samples count as two separate samples. For example, if you are supposed to run 5 TSSs a week, that should be counted as running 10 samples for that week and you should run your duplicates once a week.**

Standard Operating Procedure
- Here's that "13th Step", your SOP
- All procedures must be documented in some type of SOP
- It can be very simple but must provide the information necessary for someone who is not familiar with the test to perform it
  - Step by step instructions on how and where to collect the samples, how to run the test and how to report the values.
- It must include the QC Acceptance Criteria, the definition of a "Batch" and the minimum frequency of QC checks

Daily Laboratory Record

Month and Day _____

Operators on Duty														
Analysis		Units	00:00	02:00	04:00	06:00	08:00	10:00	12:00	14:00	16:00	18:00	20:00	22:00
Temperature	Raw	Celcius												
	Finished	Celcius												
Turbidity	Raw	NTU												
	Jar	NTU												
	Settled	NTU												
	Finished	NTU												
Chlorine, Residual	Top of Filter	mg/L												
	Lowest Plant Eff.	mg/L												
Alkalinity	Raw	mg/L												
	Jar	mg/L												
	Settled	mg/L												
	Finished	mg/L												
pH	Raw													
	Jar													
	Pre-Sed													
	Flash Mix													
	Settled													
	Clearwell													
	Finished													
Hardness	Raw	mg/L												
	Finished	mg/L												
Iron	Raw	mg/L												
	Finished	mg/L												
Manganese	Raw	mg/L												
	Finished	mg/L												
Fluoride	Raw	mg/L												
	Finished	mg/L												
Phosphate	Finished	mg/L												
TDS	Finished	mg/L												
Color	Finished	CU												
UV-254	Raw													
Odor	Finished	TON												
Carbon Dioxide	Raw	mg/L												
	Flash Mix	mg/L												
	Settled	mg/L												
	Finished	mg/L												
Langelier's	Finished													

pH

Fleming Training Center | TN Department of Environment & Conservation

pH

- One of the most important and frequently used tests in water chemistry
- A measure of the intensity of the acidic or alkaline character of a solution
- Logarithmic scale of ionic activity
  0 to 14 s.u. (standard units)
- pH values cannot be averaged

pH Theory

- pH is defined as the negative log of the molar hydrogen ion concentration in aqueous solution

$$pH = -\log [H+]$$

- pH runs from a scale of 0-14
  - $H_2O \rightarrow H^+ + OH^-$
    - $H^+ = 10^{-7}$ mol/L
    - $OH^- = \frac{10^{-7}\ mol/L}{10^{14}\ mol/L}$

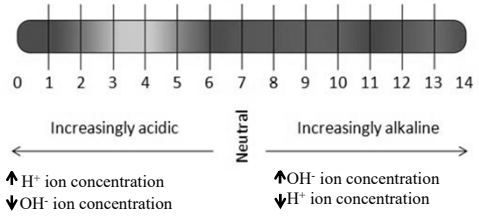
pH Measurement

- pH is typically measured with a meter and probe.
- This is an electrochemical method of analysis.

4

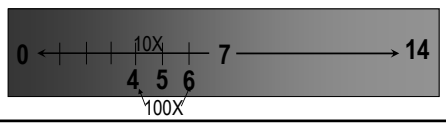
pH Theory

- Acid
  - increases the hydrogen ion (H+) concentration in a solution
- Base
  - increases the hydroxide ion (OH-) concentration in a solution



pH Scale

- pH is a negative logarithmic function
- Each decrease in pH unit = 10 times increase in acidity
  - A solution at pH 4 is 10X more acidic than a solution at pH 5
  - Solution at pH 4 is 100X more acidic than pH 6 solution
  - pH 7: $H^+ = 10^{-7}$ and $OH^- = 10^{-7}$
  - pH 4: $H^+ = 10^{-4}$ and $OH^- = 10^{-10}$



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How Does a pH Probe Work?

- Probe measures hydrogen ion concentration
  - Two electrodes in probe:
    a) Ion sensing pH half-cell
       - Glass bulb that is sensitive to H^+ ions
    b) reference half-cell
       - Glass tube filled with salt solution to complete the circuit

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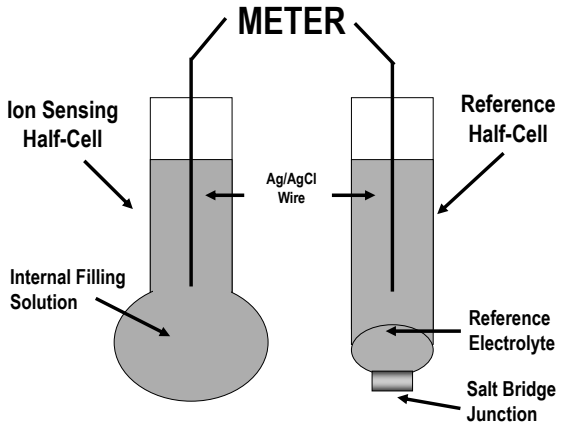

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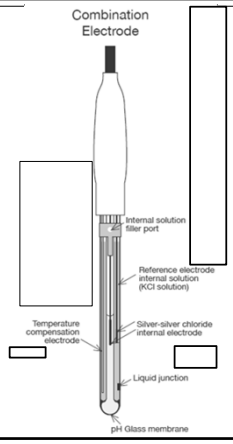

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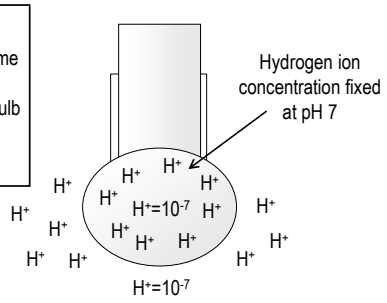
Sensing Half-Cell

pH 7 Solution

H^+ conc the same both inside and outside glass bulb

*No potential develops



TDEC - Fleming Training Center 11

Sensing Half-Cell

pH 7 Solution

H^+ conc the same both inside and outside glass bulb

*No potential develops

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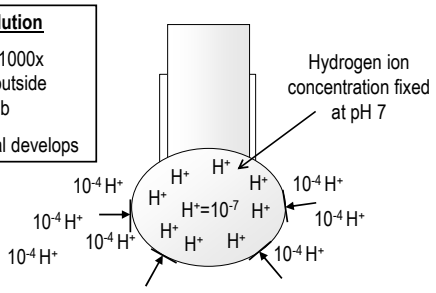
Sensing Half-Cell

pH 4 Solution

H^+ conc 1000x greater outside glass bulb

*Potential develops



Sensing Half-Cell

pH 4 Solution

H^+ conc 1000x greater outside glass bulb

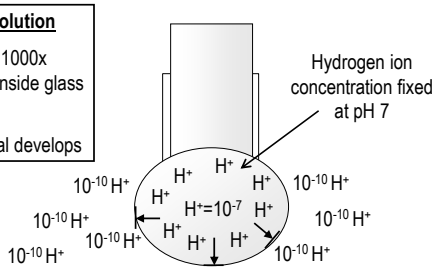
*Potential develops

Sensing Half-Cell

pH 10 Solution

H^+ conc 1000x greater inside glass bulb

*Potential develops



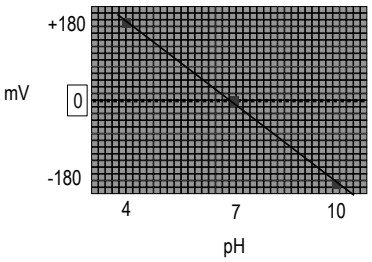
Sensing Half-Cell

pH 10 Solution

H^+ conc. 1000x greater inside glass bulb

*Potential develops

Calibration



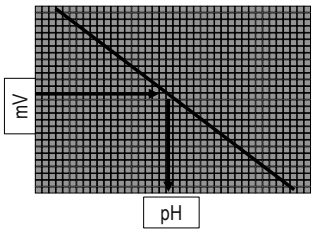
Calibration

- The optimal slope for pH is
  -58±3 mV/decade

What does this mean?

- -58 mV/decade is a generalized number
  - Each probe will have a different acceptable slope associated with it
  - When a new probe is purchased, make a note of the acceptable slope range on the bench sheet

Calibration



- A calibration curve allows the meter to convert a measured millivolt potential into a pH reading.

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Calibration

- -180 mV difference measured between pH 4 and pH 7
- pH 4 to pH 7 (3 pH units) is 1000x concentration change
- Decade = 10-fold concentration change = 1 pH unit
- -180/3 = -60 ≈ -58 mV/decade

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pH Sampling

- Holding time = 15 minutes
- Preservation = none
- Sample container = glass or plastic
- Grab sample
- Continuous monitoring possible

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pH Meter Calibration

- Follow manufacturers instructions
- Use fresh buffers (4, 7, & 10 s.u.)
- ~~Stir buffers and samples at the same speed without a vortex~~
- Constant stirring is not necessary during calibration
  - It is recommended not to stir the 10
  - Carbon dioxide mixes with water and creates carbonic acid
    - This lowers the pH and makes the 10 the most unstable
  - If mixing buffer from powder, it is a good idea to stir the standard
- Rinse and blot dry electrodes between samples and buffers
- Accurate and reproducible to within 0.1 s.u.

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pH Meter Calibration (cont.)

- Start with pH = 7.0 buffer (usually)
  - Always calibrate with at least 2 buffers, 3 recommended
  - If you only use 2 buffers, bracket the expected pH range
- Second and third buffer should be 3 s.u. different than original and brackets expected sample pH (4 or 10)
- After calibration, immerse in a known concentration - reading should be within 0.1 s.u.
- If response is accurate - read and record previous buffers as samples (pH and temperature)

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Continuous pH Monitoring EPA Method 150.2

- Immersion type electrodes easily removed from mounting:
  - Should be calibrated with two buffers that bracket expected pH and are at least 3 pH units apart
  - Adjustments made until readings are within ±0.1 s.u. of buffer value
  - Calibration must be made at least daily

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Continuous pH Monitoring EPA Method 150.2

- Immersion type electrodes <u>not</u> easily removed from mounting :
  - Indirect procedure - System must agree within 0.1 s.u. of a calibrated lab meter on an effluent sample
  - Indirect calibration once per day
  - Recalibration - System should be calibrated against two fresh buffers at least every 30 days and more often if needed

COMMON DEFICIENCIES

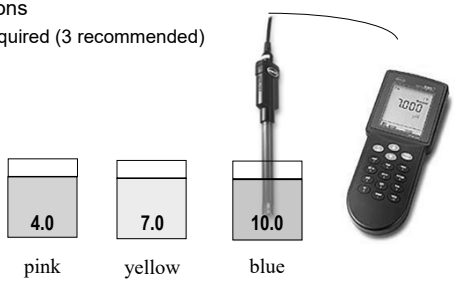
- The pH meter was calibrated using one buffer or expired buffers
- The continuous pH meter was not calibrated on a regular basis
- Buffers were left open and being reused for a week

Maintenance

- New probe
- Calibration
- Measurement/Storage
- Troubleshooting
- Cleaning

Calibrate

- Calibrate pH meters daily using two or three buffer solutions
  - 2 required (3 recommended)



Measurement

- Place probe into sample, press read button, if necessary, and wait for readings to stabilize
  - Ensure there are no bubbles on the probe tip
  - Ensure there are not encrustations on the probe
    - Often caused by the KCl storage solution
- Rinse and blot dry between measurements
  - Do not wipe
- Storage between measurements
  - pH electrode storage solution *(best)*
    - 3M KCl – same as what is in the probe to prevent loss of electrolyte
  - Sample *or* solution of similar ionic strength to sample
  - pH 7 buffer
    - Most similar to storage solution

Troubleshooting

- mV reading in pH 7 buffer
  - Should read 0 ± 30 mV in pH 7 buffer
- Response time
  - May require cleaning if slow in buffered solution
- Slope
  - Optimal slope is -58 ± 3 mV/decade (will vary from probe to probe)
- If can calibrate on 4 & 7 buffer but not 7 & 10
  - 10 buffer has probably gone bad
- If millivolts jump around when put in solution
  - Out of reference solution
    - Could be clogged or could be empty
- Storing problematic probe in pH 4 may fix problem

Cleaning

- Slow response may indicate need for cleaning
  - 1. Swish/soak probe in soapy water; rinse probe; read 7 buffer
  - 2. Soak probe in HCl; rinse probe; read 7 buffer
  - 3. Alternate soaking in dilute hydrochloric acid and dilute sodium hydroxide
    - Using less than 6 N HCl, make 1:10 dilution of HCl: H_2O
    - Rinse with deionized water
    - Condition in pH 7 buffer before use
- Read probe manual for cleaning method recommended by manufacturer

40 CFR 136

Method Update Rule
For Backwash Lagoons that have Discharge Permits
2017 Updates
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40 CFR 136 05-21-2012 Table 1B

Parameter	Methodology	EPA	Standard Methods	ASTM	USGS/AOAC/ Other
Hydrogen Ion (pH), pH units	**Electronic Measurement**	……	**4500---H^+ B-2000**	D1293-99 (A or B)	973.41, [3] I-1586-85.[2]
	Automated Electrode	150.2 (Dec. 1982)[1]	……	……	See Footnote, [21] I-2587-85[2]

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12 Quality Control Elements

1. DOC – demonstration of capability
2. MDL – method detection level
3. LRB/MB – method blank
4. LFB – laboratory fortified blank (standard)
5. LFM/LFMD – laboratory fortified matrix/duplicate (spike)
6. Internal standards, surrogate standards or tracer – ***only applies to organic analysis and radiochemistry***
7. Calibration- initial and continuing
8. Control charts or other trend analysis
9. Corrective action – root cause analysis
10. QC acceptance criteria
11. Definition of a batch (preparation and analytical)
12. Minimum frequency for conducting all QC elements
13. Unwritten 13th Step – SOP – Standard Operating Procedures need to be written and followed for all lab sampling and analyses

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pH SM4500-H^+ B – 2000 Electrometric Method

- DOC
- ICV
- CCV
- DUP
- Included in lab SOP:
  - Corrective Action
  - QC Acceptance
  - Batch Size
  - QC Frequency

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pH SM4500-H^+ B – 2000 Electrometric Measurement

- Demonstration of Capability (DOC)
  - Run buffer at least four times and compare to the limits listed in the method
  - Real people language: each operator running this test need to calibrate and analyze 4 buffers at a pH of 7
  - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
  - Recommend backup analyst do this once a year.

Read to 1/10th units only (0.0 s.u.)

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pH SM4500-H^+ B – 2000 Electrometric Measurement

* Initial Calibration
  * Calibrate per manufactures instructions with fresh buffers **daily**
* Calibration Verification
  * Read 7 buffer after analyzing samples **daily (or day of for monthly reporting purposes)**
* Duplicates of the sample
  * Run on a 5% basis, one for every 20 samples
  * Within ± 0.2 s.u.

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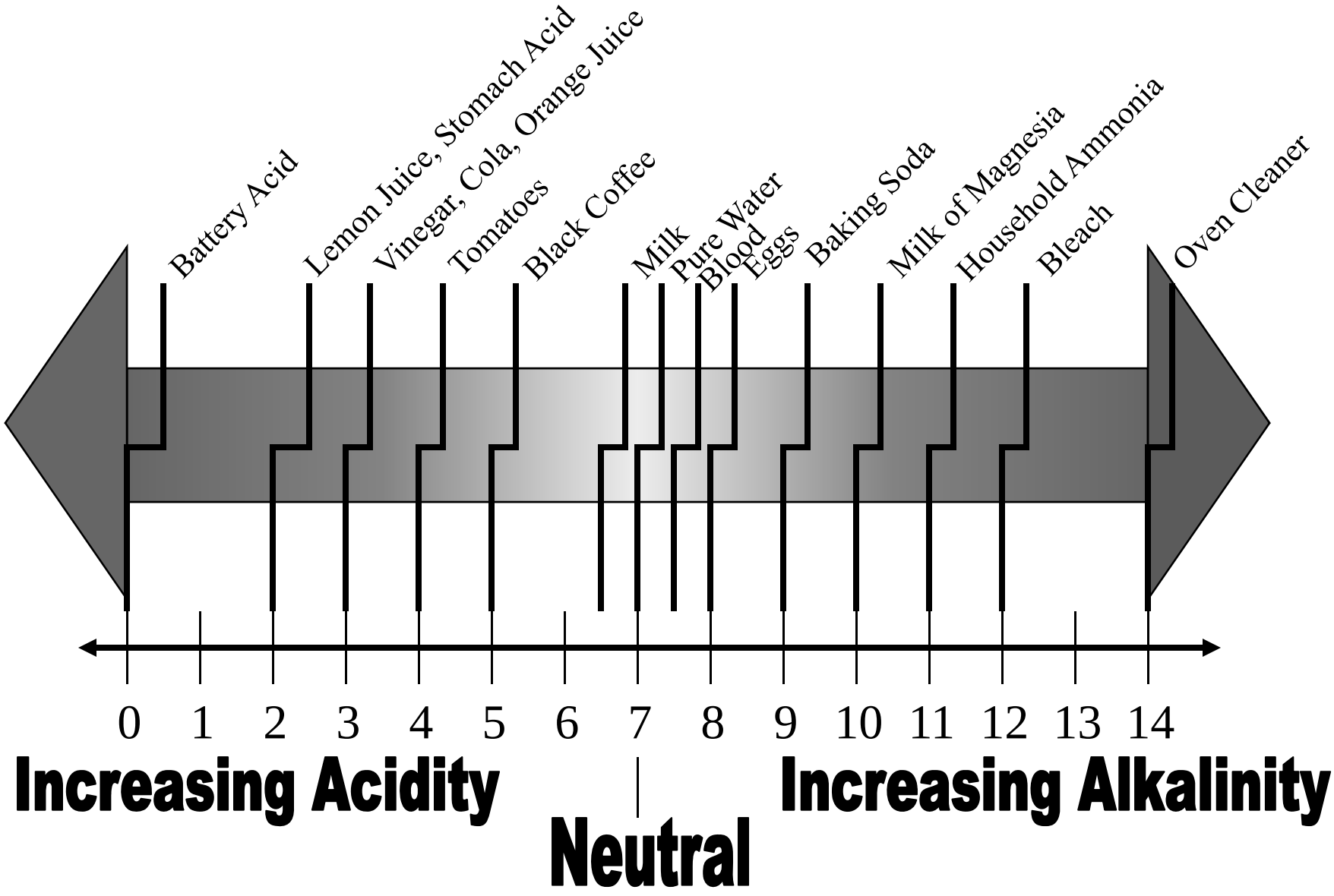
Battery Acid
Lemon Juice, Stomach Acid
Vinegar, Cola, Orange Juice
Tomatoes
Black Coffee
Milk
Pure Water
Blood
Eggs
Baking Soda
Milk of Magnesia
Household Ammonia
Bleach
Oven Cleaner
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Increasing Acidity
Neutral
Increasing Alkalinity
pH

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pH Calibration Record

Date	Time	Temp of Buffers	Slope % 95% - 105%	mV of Buffers Used			Tech	Remarks
				4	7	10		
			0.00					
			0.00					
			0.00					
			0.00					
			0.00					
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			0.00					

Slope = **|** mV pH buffer 7 – mV pH buffer 4 **|** X 100/177 OR
Slope = [the absolute value of mV (pH buffer 7 – mV pH buffer 4)] X 100/177

Month_____ **pH Meter** _____

pH, SM 4500-H^+ B, 22nd edition (2011) – Electrometric Method

Initial Demonstration of Capability (DOC)

- 4020 B.1.a. - Each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – Each operator running this test needs to calibrate instrument and analyze 4 buffers at a pH of 7**
  - **Keep a folder for each analyst, keep a copy here**
  - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
  - **Recommend backup analyst do this once a year.**
  - **Only good for that type of instrument you are using at that plant. If you have a backup instrument made by a different manufacturer or you work at another plant with a different make/model, you would need another DOC.**
    - **DOCs demonstrate you are proficient with that one instrument.**

Method Detection Limit (MDL)

- NONE

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves.
- **Real people language – calibrate daily with fresh buffers by following manufacturer's instructions.**

Method Blank

- NONE

Laboratory Fortified Blank (LFB)

- NONE

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 4020 B.2.f. – Randomly select routine samples to be analyzed twice.
  - Process duplicate sample independently through the entire sample preparation and analysis.
  - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
- **Real people language – on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) analyze 2 samples for pH, after reading one, pour out sample and start with a fresh sample**
  - **Example, grab sample in bucket and dip pH probe in twice to get a duplicate reading**

- **Target value is to get close to the first value and have a small RPD (within ± 0.1 s.u.)**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Continuing Calibration Verification (CCV)

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
  - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
  - For the calibration verification to be valid, check standards must be within +/- 0.1 pH units of its true value, and calibration blank results must not be greater than one-half the reporting level
- **Real people language – read 7 Buffer after analyzing samples daily.**

Control Charts

- NONE

Corrective Action - 1020 B.5., B.8,. & B.15.

QC Acceptance Criteria

- CCV within ± 0.1 s.u.
- Duplicates within ± 0.1 s.u.

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
  - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would suggest twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

Calculations

- NONE

4500-H^+ pH VALUE*

4500-H^+ A. Introduction

1. Principles

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment (e.g., acid–base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control) is pH-dependent. pH is used in alkalinity and carbon dioxide measurements and many other acid–base equilibria. At a given temperature the *intensity* of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity. Alkalinity and acidity are the acid- and base-neutralizing capacities of a water and usually are expressed as milligrams $CaCO_3$ per liter. Buffer capacity is the amount of strong acid or base, usually expressed in moles per liter, needed to change the pH value of a 1-L sample by 1 unit. pH as defined by Sorenson[1] is $-\log [H^+]$; it is the "intensity" factor of acidity. Pure water is very slightly ionized and at equilibrium the ion product is

$$[H^+][OH^-] = K_w = 1.01 \times 10^{-14} \text{ at } 25°C \quad (1)$$

and

$$[H^+] = [OH^-] = 1.005 \times 10^{-7}$$

where:

$[H^+]$ = activity of hydrogen ions, moles/L,
$[OH^-]$ = activity of hydroxyl ions, moles/L, and
K_w = ion product of water.

Because of ionic interactions in all but very dilute solutions, it is necessary to use the "activity" of an ion and not its molar concentration. Use of the term pH assumes that the activity of the hydrogen ion, $a_{H}{}^{+}$, is being considered. The *approximate* equivalence to molarity, $[H^+]$ can be presumed only in very dilute solutions (ionic strength <0.1).

A logarithmic scale is convenient for expressing a wide range of ionic activities. Equation 1 in logarithmic form and corrected to reflect activity is:

$$(-\log_{10} a_{H^+}) + (-\log_{10} a_{OH^-}) = 14 \quad (2)$$

or

$$\text{pH} + \text{pOH} = \text{p}K_w$$

where:

pH† = $\log_{10} a_{H+}$ and
pOH = $\log_{10} a_{OH}{}^{-}$.

Equation 2 states that as pH increases pOH decreases correspondingly and vice versa because pK_w is constant for a given temperature. At 25°C, pH 7.0 is neutral, the activities of the hydrogen and hydroxyl ions are equal, and each corresponds to an approximate activity of 10^{-7} moles/L. The neutral point is temperature-dependent and is pH 7.5 at 0°C and pH 6.5 at 60°C.

The pH value of a highly dilute solution is approximately the same as the negative common logarithm of the hydrogen ion concentration. Natural waters usually have pH values in the range of 4 to 9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals.

2. Reference

1. Sorenson, S. 1909. Über die Messung und die Bedeutung der Wasserstoff ionen Konzentration bei Enzymatischen Prozessen. *Biochem. Z.* 21:131.

* Approved by Standard Methods Committee, 2000. Editorial revisions, 2011, 2017.

† p designates $-\log_{10}$ of a number.

4500-H^+ B. Electrometric Method

1. General Discussion

a. Principle: The basic principle of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The hydrogen electrode consists of a platinum electrode across which hydrogen gas is bubbled at a pressure of 101 kPa. Because of difficulty in its use and the potential for poisoning the hydrogen electrode, the glass electrode commonly is used. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by extrapolation.

https://doi.org/10.2105/SMWW.2882.082

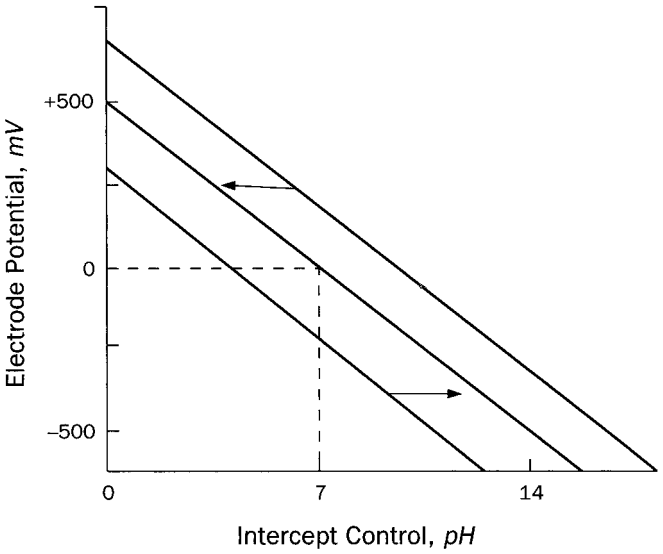

Figure 4500-H$^+$:1. Electrode potential vs. pH. Intercept control shifts response curve laterally.

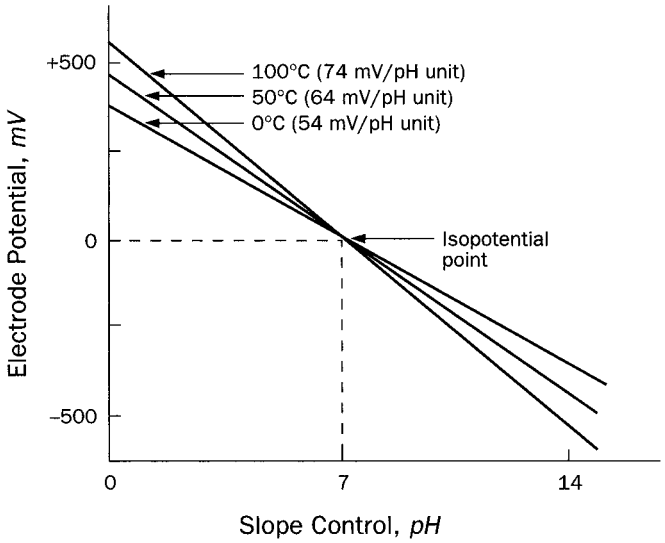

Figure 4500-H$^+$:2. Typical pH electrode response as a function of temperature.

Because single ion activities, such as a_{H^+}, cannot be measured, pH is defined operationally on a potentiometric scale. The pH measuring instrument is calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using National Institute of Standards and Technology (NIST) buffers having assigned values so:

$$pH_B = -\log_{10} a_{H^+}$$

where:

pH_B = assigned pH of NIST buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$pH_x = pH_B \pm \frac{F(E_x - E_s)}{2.303\,RT}$$

where:

pH_x = potentiometrically measured sample pH,
F = Faraday: 9.649×10^4 coulomb/mole,
E_x = sample emf, V,
E_s = buffer emf, V,
R = gas constant; 8.314 joule/(mole K), and
T = absolute temperature, K.

NOTE: Although the equation for pH_x appears in the literature with a plus sign, the sign of emf readings in millivolts for most pH meters manufactured in the United States is negative. The choice of negative sign is consistent with the IUPAC Stockholm convention concerning the sign of electrode potential.[1,2]

The activity scale gives values that are higher than those on Sorenson's scale by 0.04 units:

$$\text{pH (activity)} = \text{pH (Sorenson)} + 0.04$$

The equation for pH_x assumes that the emf of the cells containing the sample and buffer is due solely to hydrogen ion activity unaffected by sample composition. In practice, samples will have varying ionic species and ionic strengths, both affecting H^+ activity. This imposes an experimental limitation on pH measurement; thus, to obtain meaningful results, the differences between E_x and E_s should be minimal. Samples must be dilute aqueous solutions of simple solutes ($<0.2M$). (Choose buffers to bracket the sample.) Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high-ionic-strength solutions.

b. Interferences: The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductants, or high salinity, except for a sodium error at pH >10. Reduce this error by using special "low sodium error" electrodes.

pH measurements are affected by temperature in two ways: mechanical effects that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes. In the first instance, the Nernstian slope increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long-term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures.

Always report temperature at which pH is measured.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:I.

2. Apparatus

a. pH meter consisting of potentiometer, a glass electrode, a reference electrode, and a temperature-compensating device. A circuit is completed through the potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolts and some have scale expan-

https://doi.org/10.2105/SMWW.2882.082

TABLE 4500-H$^+$:I. PREPARATION OF pH STANDARD SOLUTIONS[3]

Standard Solution (molality)	pH at 25°C	Weight of Chemicals Needed/1000 mL Pure Water at 25°C
Primary standards:		
Potassium hydrogen tartrate (saturated at 25°C)	3.557	> 7 g $KHC_4H_4O_6$*
0.05 potassium dihydrogen citrate	3.776	11.41 g $KH_2C_6H_5O_7$
0.05 potassium hydrogen phthalate	4.004	10.12 g $KHC_8H_4O_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.863	3.387 g KH_2PO_4 + 3.533 g Na_2HPO_4†
0.008 695 potassium dihydrogen phosphate + 0.030 43 disodium hydrogen phosphate	7.415	1.179 g KH_2PO_4 + 4.303 g Na_2HPO_4†
0.01 sodium borate decahydrate (borax)	9.183	3.80 g $Na_2B_4O_7 \cdot 10H_2O$†
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.014	2.092 g $NaHCO_3$ + 2.640 g Na_2CO_3
Secondary standards:		
0.05 potassium tetroxalate dihydrate	1.679	12.61 g $KH_3C_4O_8 \cdot 2H_2O$
Calcium hydroxide (saturated at 25°C)	12.454	> 2 g $Ca(OH)_2$*

* Approximate solubility.
† Prepare with freshly boiled and cooled distilled water (carbon-dioxide-free).

sion that permits reading to 0.001 pH unit, but most instruments are not that precise.

For routine work, use a pH meter accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature-compensation adjustment.

Although manufacturers provide operating instructions, the use of different descriptive terms may be confusing. For most instruments, there are two controls: intercept (set buffer, asymmetry, standardize) and slope (temperature, offset); their functions are shown diagramatically in Figures 4500-H$^+$:1 and 2. The intercept control shifts the response curve laterally to pass through the isopotential point with no change in slope. This permits bringing the instrument on scale (0 mV) with a pH 7 buffer that has no change in potential with temperature.

The slope control rotates the emf/pH slope about the isopotential point (0 mV/pH 7). To adjust slope for temperature without disturbing the intercept, select a buffer that brackets the sample with pH 7 buffer and adjust slope control to pH of this buffer. The instrument will indicate correct millivolt change per unit pH at the test temperature.

b. Reference electrode consisting of a half cell that provides a constant electrode potential. Commonly used are calomel and silver: silver-chloride electrodes. Either is available with several types of liquid junctions.

The liquid junction of the reference electrode is critical because at this point the electrode forms a salt bridge with the sample or buffer and a liquid junction potential is generated that in turn affects the potential produced by the reference electrode. Reference electrode junctions may be annular ceramic, quartz, or asbestos fiber, or the sleeve type. The quartz type is most widely used. The asbestos fiber type is not recommended for strongly basic solutions. Follow the manufacturer's recommendation on use and care of the reference electrode.

Refill nonsealed electrodes with the correct electrolyte to proper level and make sure junction is properly wetted.

c. Glass electrode: The sensor electrode is a bulb of special glass containing a fixed concentration of HCl or a buffered chloride solution in contact with an internal reference electrode. Upon immersion of a new electrode in a solution, the outer bulb surface becomes hydrated and exchanges sodium ions for hydrogen ions to build up a surface layer of hydrogen ions. This, together with the repulsion of anions by fixed, negatively charged silicate sites, produces at the glass–solution interface a potential that is a function of hydrogen ion activity in solution.

Several types of glass electrodes are available. Combination electrodes incorporate the glass and reference electrodes into a single probe. Use a "low sodium error" electrode that can operate at high temperatures for measuring pH > 10 because standard glass electrodes yield erroneously low values. For measuring pH <1 standard glass electrodes yield erroneously high values; use liquid membrane electrodes instead.

d. Beakers: Preferably use polyethylene or TFE* beakers.

e. Stirrer: Use either a magnetic, TFE-coated stirring bar or a mechanical stirrer with inert plastic-coated impeller.

f. Flow chamber: Use for continuous flow measurements or for poorly buffered solutions.

3. Reagents

a. General preparation: Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work by weighing the amounts of chemicals specified in Table 4500-H$^+$:I, dissolving in distilled water at 25°C, and diluting to 1000 mL. This is particularly important for borate and carbonate buffers.

Boil and cool distilled water having a conductivity of less than 2 μmhos/cm. To 50 mL, add 1 drop of saturated KCl solution suitable for reference electrode use. If the pH of this test solution is between 6.0 and 7.0, use it to prepare all standard solutions.

Dry KH_2PO_4 at 110 to 130°C for 2 h before weighing but do not heat unstable hydrated potassium tetroxalate above 60°C nor dry the other specified buffer salts.

* Teflon, or equivalent

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TABLE 4500-H$^+$:II. STANDARD pH VALUES[3]

Temperature °C	Primary Standards							Secondary Standards	
	Tartrate (Saturated)	Citrate (0.05*M*)	Phthalate (0.05*M*)	Phosphate (1:1)	Phosphate (1:3.5)	Borax (0.01*M*)	Bicarbonate-Carbonate (0.025*M*)	Tetroxalate (0.05*M*)	Calcium Hydroxide (Saturated)
0			4.003	6.982	7.534	9.460	10.321	1.666	
5			3.998	6.949	7.501	9.392	10.248	1.668	
10			3.996	6.921	7.472	9.331	10.181	1.670	
15			3.996	6.898	7.449	9.276	10.120	1.672	
20			3.999	6.878	7.430	9.227	10.064	1.675	
25	3.557	3.776	4.004	6.863	7.415	9.183	10.014	1.679	12.454
30	3.552		4.011	6.851	7.403	9.143	9.968	1.683	
35	3.549		4.020	6.842	7.394	9.107	9.928	1.688	
37			4.024	6.839	7.392	9.093			
40	3.547		4.030	6.836	7.388	9.074	9.891	1.694	
45	3.547		4.042	6.832	7.385	9.044	9.859	1.700	
50	3.549		4.055	6.831	7.384	9.017	9.831	1.707	
55	3.554		4.070					1.715	
60	3.560		4.085					1.723	
70	3.580		4.12					1.743	
80	3.609		4.16					1.766	
90	3.650		4.19					1.792	
95	3.674		4.21					1.806	

Although ACS-grade chemicals generally are satisfactory for preparing buffer solutions, use certified materials available from the National Institute of Standards and Technology when the greatest accuracy is required. For routine analysis, use commercially available buffer tablets, powders, or solutions of tested quality. In preparing buffer solutions from solid salts, ensure complete solution.

As a rule, select and prepare buffer solutions classed as primary standards in Table 4500-H$^+$:I; reserve secondary standards for extreme situations encountered in wastewater measurements. Consult Table 4500-H$^+$:II for accepted pH of standard buffer solutions at temperatures other than 25°C. In routine use, store buffer solutions and samples in polyethylene bottles. Replace buffer solutions every 6 months.

b. Saturated potassium hydrogen tartrate solution: Shake vigorously an excess (5 to 10 g) of finely crystalline $KHC_4H_4O_6$ with 100 to 300 mL distilled water at 25°C in a glass-stoppered bottle. Separate clear solution from undissolved material by decantation or filtration. Preserve for 2 months or more by adding one thymol crystal (8 mm diam) per 200 mL solution.

c. Saturated calcium hydroxide solution: Calcine a well-washed, low-alkali grade $CaCO_3$ in a platinum dish by igniting for 1 h at 1000°C. Cool, hydrate by slowly adding distilled water with stirring, and heat to boiling. Cool, filter, and collect solid $Ca(OH)_2$ on a fritted glass filter of medium porosity. Dry at 110°C, cool, and pulverize to uniformly fine granules. Vigorously shake an excess of fine granules with distilled water in a stoppered polyethylene bottle. Let temperature come to 25°C after mixing. Filter supernatant under suction through a sintered glass filter of medium porosity and use filtrate as the buffer solution. Discard buffer solution when atmospheric CO_2 causes turbidity to appear.

d. Auxiliary solutions: 0.1*N* NaOH, 0.1*N* HCl, 5*N* HCl (dilute five volumes 6*N* HCl with one volume distilled water), and acid potassium fluoride solution (dissolve 2 g KF in 2 mL conc H_2SO_4 and dilute to 100 mL with distilled water).

4. Procedure

a. Instrument calibration: In each case, follow manufacturer's instructions for pH meter and for storage and preparation of electrodes for use. Recommended solutions for short-term storage of electrodes vary with type of electrode and manufacturer, but generally have a conductivity greater than 4000 μmhos/cm. A pH 4 buffer is best for the single glass electrode and saturated KCl is preferred for a calomel and Ag/AgCl reference electrode. Saturated KCl is the preferred solution for a combination electrode. Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

Before use, remove electrodes from storage solution, rinse, blot dry with a soft tissue, place in initial buffer solution, and set the isopotential point (4500-H$^+$.B.2*a*). Select a second buffer within 2 pH units of sample pH and bring sample and buffer to same temperature, which may be the room temperature; a fixed temperature, such as 25°C; or the temperature of a fresh sample. Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Record temperature of measurement and adjust temperature dial on meter so meter indicates pH value of buffer at test temperature (this is a slope adjustment).

Use the pH value listed in the tables for the buffer used at the test temperature. Remove electrodes from second buffer, rinse thoroughly with distilled water and dry electrodes as indicated above. Immerse in a third buffer below pH 10, approximately

3 pH units different from the second; the reading should be within 0.1 unit for the pH of the third buffer. If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or potentiometer (4500-H$^+$.B.5*a* and *b*).

The purpose of standardization is to adjust the response of the glass electrode to the instrument. When only occasional pH measurements are made, standardize instrument before each measurement. When frequent measurements are made and the instrument is stable, standardize less frequently. If sample pH values vary widely, standardize for each sample with a buffer having a pH within 1 to 2 pH units of the sample.

b. Sample analysis: Establish equilibrium between electrodes and sample by stirring sample to ensure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes after cleaning by dipping them into sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

5. Troubleshooting

a. Potentiometer: To locate trouble source, disconnect electrodes and, using a short-circuit strap, connect reference electrode terminal to glass electrode terminal. Observe change in pH when instrument calibration knob is adjusted. If potentiometer is operating properly, it will respond rapidly and evenly to changes in calibration over a wide scale range. A faulty potentiometer will fail to respond, will react erratically, or will show a drift upon adjustment. Switch to the millivolt scale on which the meter should read zero. If inexperienced, do not attempt potentiometer repair other than maintenance as described in instrument manual.

b. Electrodes: If potentiometer is functioning properly, look for the instrument fault in the electrode pair. Substitute one electrode at a time and cross-check with two buffers that are about 4 pH units apart. A deviation greater than 0.1 pH unit indicates a faulty electrode. Glass electrodes fail because of scratches, deterioration, or accumulation of debris on the glass surface. Rejuvenate electrode by alternately immersing it three times each in 0.1*N* HCl and 0.1*N* NaOH. If this fails, immerse tip in KF solution for 30 s. After rejuvenation, soak in pH 7.0 buffer overnight. Rinse and store in pH 7.0 buffer. Rinse again with distilled water before use. Protein coatings can be removed by soaking glass electrodes in a 10% pepsin solution adjusted to pH 1 to 2.

To check reference electrode, oppose the emf of a questionable reference electrode against another one of the same type that is known to be good. Using an adapter, plug good reference electrode into glass electrode jack of potentiometer; then plug questioned electrode into reference electrode jack. Set meter to read millivolts and take readings with both electrodes immersed in the same electrolyte (KCl) solution and then in the same buffer solution. The millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used (i.e., silver:silver-chloride against calomel or vice versa) the reading will be 44 ± 5 mV for a good reference electrode.

Reference electrode troubles generally are traceable to a clogged junction. Interruption of the continuous trickle of electrolyte through the junction causes increase in response time and drift in reading. Clear a clogged junction by applying suction to the tip or by boiling tip in distilled water until the electrolyte flows freely when suction is applied to tip or pressure is applied to the fill hole. Replaceable junctions are available commercially.

6. Precision and Bias

By careful use of a laboratory pH meter with good electrodes, a precision of ±0.02 pH unit and an accuracy of ±0.05 pH unit can be achieved. However, ±0.1 pH unit represents the limit of accuracy under normal conditions, especially for measurement of water and poorly buffered solutions. For this reason, report pH values to the nearest 0.1 pH unit. A synthetic sample of a Clark and Lubs buffer solution of pH 7.3 was analyzed electrometrically by 30 laboratories with a standard deviation of ±0.13 pH unit.

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pH

USEPA Electrode Method

Method 8156
pH electrode

Scope and application: For drinking water[1], wastewater[2] and process water.

[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

Instrument specific information

This procedure is applicable to the meters and probes that are shown in Table 1. Procedures for other meters and probes can be different.

Table 1 Instrument-specific information

Meter	Probe
HQ1100 and HQ11d portable one input, pH/ORP HQ4100, HQ2100 and HQ30d portable one input, multi-parameter HQ4200, HQ2200 and HQ40d portable two input, multi-parameter HQ4300 portable three input, multi-parameter HQ411d benchtop one input, pH/mV HQ430d benchtop one input, multi-parameter HQ440d benchtop two input, multi-parameter	Intellical PHC101, PHC201, PHC281 or PHC301 pH
Sension+ MM156 portable pH/EC/DO	Sension+ 5049 multi-parameter
Sension+ pH1 portable pH Sension+ MM110 portable pH/ORP Sension+ MM150 portable pH/ORP/EC	Sension+ 5050T, 5051T or 5052T combination pH Sension+ 5045, 5048 or 5059 multi-parameter
Sension+ pH3 lab pH Sension+ pH31 GLP lab pH Sension+ MM340 lab two input, pH/mV/ISE Sension+ MM374 lab two input, pH/mV/EC/ISE Sension+ MM378 lab two input, pH/ISE/EC/DO	Sension+ 5010T, 5011T, 5014T or 5021T combination pH

Before starting

Refer to the meter documentation for meter settings and operation. Refer to probe documentation for probe preparation, maintenance and storage information.
Prepare the probe before initial use. Refer to probe documentation.
When an Intellical probe is connected to an HQ meter or an HQd meter, the meter automatically identifies the measurement parameter and is prepared for use.
Condition the electrode for the best response time. To condition the electrode, soak the electrode for several minutes in a solution that has almost the same pH and ionic strength as the sample.
Calibrate the probe before initial use. Refer to Calibration procedure on page 3.
For rugged electrodes, it may be necessary to remove the shroud before measurement and calibration.
Air bubbles under the sensor tip can cause slow response or measurement errors. To remove the bubbles, carefully shake the probe.
To save data automatically, set the measurement mode to Press to Read or Interval. When the measurement mode is Continuous, select Store to save data manually.

Rinse the electrode between measurements to prevent contamination.
Keep the electrode in a pH storage solution when not in use. Refer to the probe documentation.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.
This procedure is specified for the HQ meters and HQd meters. The Sension+ meters can be used, but the menus and navigation will be different.

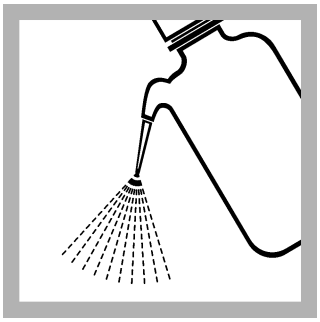
Items to collect

Description	Quantity
Beaker or sample containers	3
Wash bottle with deionized water	1
pH buffers (4.0, 7.0, 10.0)	3

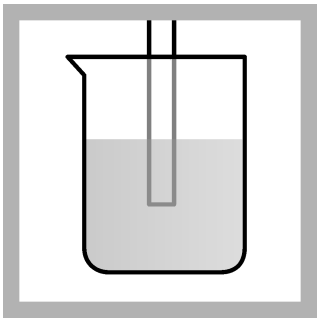
Sample collection

- Analyze the samples immediately. The samples cannot be preserved for later analysis.
- Collect samples in clean glass or plastic bottles.

Test procedure

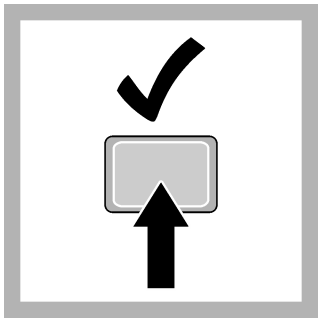


1. Rinse the probe with deionized water. Dry the probe with a lint-free cloth.

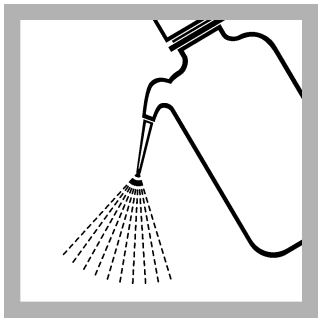


2. Laboratory test: Put the probe in a beaker that contains the solution. Do not let the probe touch the stir bar, bottom or sides of the container. Remove the air bubbles from under the probe tip. Stir the sample at a slow to moderate rate.
Field test: Put the probe in the sample. Move the probe up and down to remove bubbles from the electrode.

Make sure to put the temperature sensor fully in the sample.

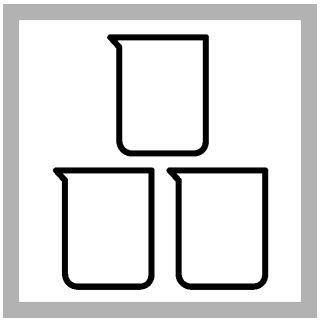


3. Push **Read**. A progress bar is shown. When the measurement is stable, the lock icon is shown.



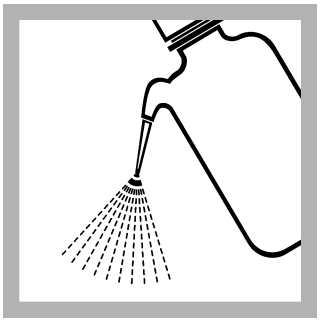
4. Rinse the probe with deionized water. Dry the probe with a lint-free cloth.

Calibration procedure

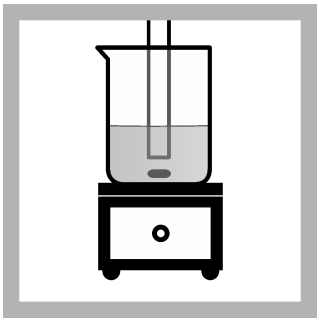


1. Prepare two or three fresh buffer solutions in separate beakers. If two buffers are used, use a 7.0 and a 4.0 or a 7.0 and a 10.0 pH buffer solution.

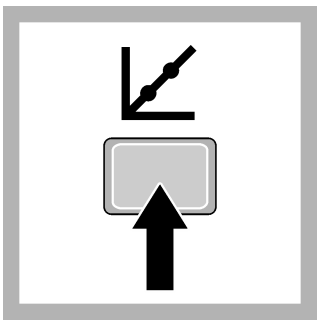
2. Add a stir bar and put the beaker on a magnetic stirrer. Stir at a moderate rate.



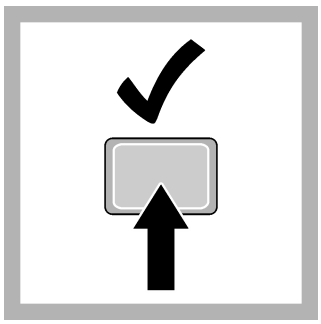
3. Rinse the probe with deionized water. Dry the probe with a lint-free cloth.



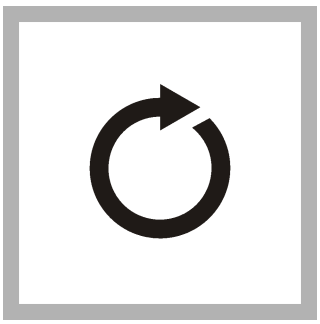
4. Put the probe in the solution. Do not let the probe touch the stir bar, bottom or sides of the container. Remove the air bubbles from under the probe tip.



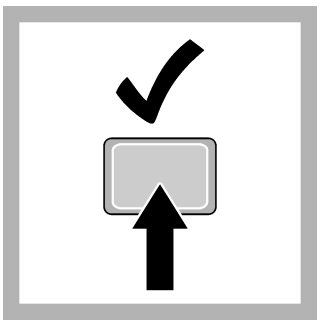
5. Push **Calibrate**. The standard solution value is shown.



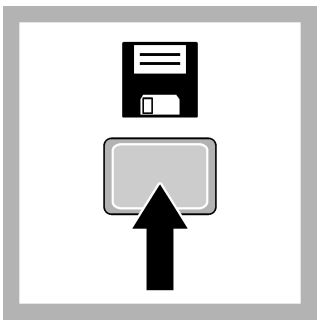
6. Push **Read**. A progress bar is shown. When the measurement is stable, the lock icon is shown.



7. Measure the remaining buffer solutions.



8. Push **Done**. A calibration summary is shown when the minimum number of calibration standards are measured.



9. Push **Store** to accept the calibration.

Low ionic strength or high-purity water measurements

NOTICE

Do not keep the probe in LIS samples for a long period of time because this can decrease the probe life. Put the probe in electrode storage solution or 3 M KCl when LIS measurements are complete.

Low ionic strength (LIS) solutions have very low buffering capacity and absorb carbon dioxide from the air. When a sample absorbs carbon dioxide from the atmosphere, carbonic acid forms. Carbonic acid decreases the sample pH, which causes inaccurate

readings. One solution to this problem is to measure the sample in a low volume, airtight sample chamber such as a low ionic strength chamber.

Use refillable or platinum series electrodes for measurement of pH in LIS or high purity waters.

Before an LIS sample is measured, condition the probe as follows:

1. Soak the probe in a solution equivalent to the sample in ionic strength and pH for 10 to 15 minutes.
2. Rinse the probe with deionized water.
3. Dry the probe with a soft paper towel.

Between measurements, keep the probe in the sample or a neutral LIS solution (e.g., tap water) for a maximum of 2 hours.

Interferences

The sodium error is low but increases at pH values that are higher than pH 11. The acid error is negligible. Refer to the electrode or the meter documentation.

Accuracy check

Slope test

The electrode operation is satisfactory when the calibration slope is within the specified range (typically –58 mV (±3) at 25 °C).

Calibration accuracy

Measure the pH of a fresh buffer solution. A calibration is satisfactory when the measured pH value agrees with the known pH value of the buffer solution.

Clean the probe

Clean the probe when:

- Drifting/inaccurate readings occur as a result of contamination on the sensing element or incorrect storage conditions.
- Slow response time occurs as a result of contamination on the sensing element.
- The slope is out of range as a result of contamination on the sensing element.

For general contamination, complete the steps that follow.

1. Rinse the probe with deionized water. Blot dry with a lint-free cloth.
2. Soak the glass bulb for 12 to 16 hours in Hach Electrode Cleaning Solution.
3. Rinse or soak the probe for 1 minute in deionized water.
4. Soak the probe in pH 4 buffer for up to 20 minutes, then rinse with deionized water.
5. Blot dry with a lint-free cloth.
6. If harsh contaminants are attached to the probe, polish the probe tip with a soft cloth or cotton swab to remove the contaminants.
7. Soak for up to 20 minutes in pH 4 buffer, then rinse with deionized water.

Method performance

The accuracy of the measurements is dependent on many factors that are related with the overall system, which includes the meter, the probe and calibration solutions. Refer to the meter or probe documentation for more information.

Summary of method

A combination pH electrode develops a potential at the glass/liquid interface. At a constant temperature, this potential varies linearly with the pH of the solution.

The pH is 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. The sample pH can change when carbon dioxide is absorbed from the atmosphere. In water that has a high conductivity, the buffer capacity is typically high and the pH does not change much.

Consumables and replacement items

HQ meters, HQd meters and probes

Description	Unit	Item no.
HQ1110 portable one input, pH/ORP meter	each	LEV015.53.1110A
HQ2100 portable one input, multi-parameter meter	each	LEV015.53.2100A
HQ2200 portable two input, multi-parameter meter	each	LEV015.53.2200A
HQ4100 portable one input, multi-parameter meter	each	LEV015.53.4100A
HQ4200 portable two input, multi-parameter meter	each	LEV015.53.4200A
HQ4300 portable three input, multi-parameter meter	each	LEV015.53.4300A
HQ411d benchtop one input, pH/mV meter	each	HQ411D
HQ430d benchtop one input, multi-parameter meter	each	HQ430D
HQ440d benchtop two input, multi-parameter meter	each	HQ440D
Intellical pH gel probe, standard with 1 m cable	each	PHC10101
Intellical pH gel probe, standard with 3 m cable	each	PHC10103
Intellical pH gel probe, rugged with 5 m cable	each	PHC10105
Intellical pH gel probe, rugged with 10 m cable	each	PHC10110
Intellical pH gel probe, rugged with 15 m cable	each	PHC10115
Intellical pH gel probe, rugged with 30 m cable	each	PHC10130
Intellical pH gel probe, standard with 1 m cable	each	PHC20101
Intellical pH gel probe, standard with 3 m cable	each	PHC20103
Intellical pH gel probe, ultra with 1 m cable	each	PHC28101
Intellical pH gel probe, ultra with 3 m cable	each	PHC28103
Intellical pH liquid probe, standard with 1 m cable	each	PHC30101
Intellical pH liquid probe, standard with 3 m cable	each	PHC30103

Sension+ meters and probes

Description	Unit	Item no.
Sension+ pH3 lab pH meter	each	LPV2010T.97.002
Sension+ pH31 GLP lab pH meter	each	LPV2110T.97.002
Sension+ MM340 lab two input, pH/mV/ISE meter	each	LPV2200.97.0002
Sension+ MM374 lab two input, pH/mV/EC/ISE meter	each	LPV4110.97.0002
Sension+ MM378 lab two input, pH/ISE/EC/DO meter	each	LPV4130.97.0002
Sension+ 5010T combination pH probe	each	LZW5010T.97.002
Sension+ 5011T combination pH probe	each	LZW5011T.97.002
Sension+ 5014T combination pH probe	each	LZW5014T.97.002
Sension+ 5021T combination pH probe	each	LZW5021T.97.002
Sension+ 5050T combination pH probe	each	LZW5050T.97.002
Sension+ 5051T combination pH probe	each	LZW5051T.97.002
Sension+ 5052T combination pH probe	each	LZW5052T.97.002
Sension+ 5045 multi-parameter probe	each	LZW5045.97.0002

Sension+ meters and probes (continued)

Description	Unit	Item no.
Sension+ 5048 multi-parameter probe	each	LZW5048.97.0002
Sension+ 5049 multi-parameter probe	each	LZW5049.97.0002
Sension+ 5059 multi-parameter probe	each	LZW5059.97.0002

Recommended standards

Description	Unit	Item no.
pH 4.01 buffer solution, Singlet one-use packets, 20 mL each	20/pkg	2770020
pH 7.00 buffer solution, Singlet one-use packets, 20 mL each	20/pkg	2770120
pH 10.01 buffer solution, Singlet one-use packets, 20 mL each	20/pkg	2770220
pH 4.01 and pH 7.00 buffer solution kit, Singlet one-use packets, 20 mL each	2 x 10/pkg	2769920
pH 7.00 and 10.01 buffer solution kit, Singlet one-use packets, 20 mL each	2 x 10/pkg	2769820
pH color-coded buffer solution kit (NIST), 500 mL, includes:	1	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:		
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 (77 °F)	500 mL	S11M001
pH 4.005 ± 0.010 at 25 °C (77 °F)	500 mL	S11M002
pH 6.865 ± 0.010 at 25 °C (77 °F)	500 mL	S11M003
pH 7.000 ± 0.010 at 25 °C (77 °F)	500 mL	S11M004
pH 9.180 ± 0.010 at 25 °C (77 °F)	500 mL	S11M006
pH 10.012 ± 0.010 at 25 °C (77 °F)	500 mL	S11M007
pH 12.45 ± 0.05 at 25 °C (77 °F)	500 mL	S11M008
pH buffer 1.09, technical	500 mL	S11M009
pH buffer 4.65, technical	500 mL	S11M010
pH buffer 9.23, technical	500 mL	S11M011

Accessories

Description	Unit	Item no.
Beaker, polypropylene, 50 mL, low form	each	108041
Beaker, polypropylene, 100-mL	each	108042
Bottle, wash, 500 mL	each	62011
Stir bar, magnetic, 2.2 x 0.5 cm (7/8 x 3/16 in.)	each	4531500
Stirrer, electromagnetic, 120 VAC, with electrode stand	each	4530001
Stirrer, electromagnetic, 230 VAC, with electrode stand	each	4530002

Accessories (continued)

Description	Unit	Item no.
Sample bottle with screw-top cap, polypropylene, 500-mL	each	2758101
Water, deionized	4 L	27256

Month _____

pH Meter _____ **Method # _____**

pH Lab

Date	Time	Sample Location	Measured pH	Date of Last Calibration	Analyst

Density and Specific Gravity

Fleming Training Center | TN Department of Environment & Conservation

•1

Density

- Weight per unit volume
  - solids and gases expressed in lb/ft^3
  - liquids measured in lb/gal or lb/ft^3
- Density of water varies slightly with temperature and pressure
- Density of gases changes significantly with changes in temperature and pressure

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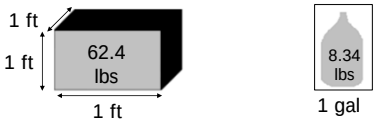
Density of Water



The density of water is
62.4 lbs/ft^3
or
8.34 lbs/gal

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Specific Gravity

- Compares density of a substance to a standard density
- Does not have units
- For solids and liquids
  - compare to standard density of water
    - 62.4 lb/ft^3
    - 8.34 lb/gal

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Example #1

- The density of SAE motor oil is 56 lb/ft^3. Find the specific gravity.

$$specific\ gravity = \frac{density\ of\ substance}{density\ of\ water}$$

$$specific\ gravity = \frac{56\ {}^{lb}/_{ft^3}}{62.4\ {}^{lb}/_{ft^3}}$$

$$specific\ gravity = 0.90$$

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Example #2

- A sample of liquid alum has a density of 11.2 lb/gal. What is the specific gravity?

$$specific\ gravity = \frac{density\ of\ substance}{density\ of\ water}$$

$$specific\ gravity = \frac{11.2\ {}^{lb}/_{gal}}{8.34\ {}^{lb}/_{gal}}$$

$$specific\ gravity = 1.34$$

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Specific Gravity of Gases

Compare to standard density of air:
0.075 lb/ft^3

Example #3

- The density chlorine gas is 0.187 lb/ft^3. Find the specific gravity.

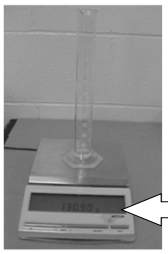
$$specific\ gravity = \frac{density\ of\ substance}{density\ of\ air}$$

$$specific\ gravity = \frac{0.187\ {}^{lb}/_{ft^3}}{0.075\ {}^{lb}/_{ft^3}}$$

$$specific\ gravity = 2.49$$

Measuring Specific Gravity

Chemical Verification

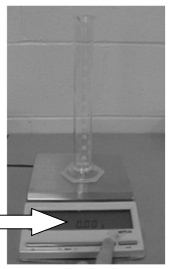


- Place 100 mL graduated cylinder on a scale

130.90 g

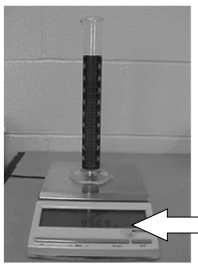
Chemical Verification

- Tare the scale so it reads zero with the cylinder on it.



0.00 g

Chemical Verification



- Weigh 100 mL of your sample or chemical.

99.09 g

Chemical Verification

- Find the specific gravity of a substance in which 100 mL weighs 99.09 grams.

$$specific\ gravity = \frac{weight\ of\ 100\ mL\ of\ sol'n, grams}{100\ grams}$$

$$specific\ gravity = \frac{99.09\ g}{100\ g}$$

$$specific\ gravity = 0.99$$

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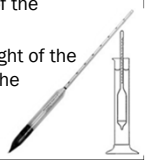
Hydrometer

- A hydrometer is an instrument whose function is based on Archimedes principle.
- This principle states that a body (the hydrometer) immersed in a fluid is buoyed up by a force equal to the weight of the displaced fluid.
- The hydrometer measures the weight of the liquid displaced by the volume of the hydrometer.



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Hydrometer

- The greater the density, the tighter or closer the molecules are packed inside the substance.
- Therefore, the greater the density / specific gravity of a liquid the higher a hydrometer will be buoyed by it.

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Hydrometer

- Fill your hydrometer jar about ¾ with the liquid you wish to test
- Insert the hydrometer slowly. Do not drop it in!
- Now give it a spin with your thumb and index finger, this will dislodge any bubbles that may have formed
- Once the hydrometer comes to a rest, observe the plane of the liquid surface
  - Your eye must be horizontal to this plane
- The point at which this line cuts the hydrometer scale is your reading

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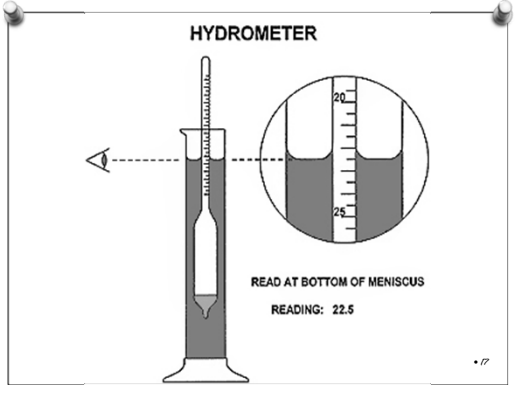

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DENSITY & SPECIFIC GRAVITY

Density: Weight per unit volume.

2 ways to express density: _____

_____

Specific gravity: Density of any substance compared to a "standard density."

Standard density of water: _____ lb/gal

_____ lb/ft^3

1. Find the specific gravity for rock granite if the density is 162 lbs/ft^3.

2. Find the specific gravity for SAE 30 motor oil if the density is 56 lbs/ft^3.

3. Find the specific gravity of dry alum if the density is 65 lbs/ft^3.

4. Find the specific gravity for liquid alum that weighs 11.07 lbs/gal.

5. Find the specific gravity for fluorosilicic acid that weighs 10.5 lbs/gal.

6. Find the specific gravity for ferric sulfate that weighs 12.34 lbs/gal.

7. Find the density (lbs/ft^3) of a certain oil that has a S.G. of 0.92.

8. Find the density (lbs/gal) of ferric chloride that has a S.G. of 1.140.

9. Find the density (lbs/gal) of caustic soda that has a S.G. of 1.530.

10. Find the density (lbs/ft^3) of potassium permanganate that has a S.G. of 1.522.

11. A tank holds 1,240 gallons of a certain liquid. The specific gravity is 0.93. How many pounds of liquid are in the tank?

12. Pump rate desired: 25 gpm
Liquid weight: 74.9 lbs/ft^3
How many pounds of liquid can be pumped per day?

13. A certain pump delivers 23 gallons per minute.
    A. How many lbs of water does the pump deliver in 1 minute?
    B. How many lbs/min will the pump deliver if the liquid weighs 71.9 lbs/ft^3?

14. A certain pump delivers 14 gallons per minute.
    A. How many lbs of water does the pump deliver in 24 hours?
    B. How many lbs/day will the pump deliver if the liquid weighs 8.1 lbs/gal?

15. Compare the density of chlorine gas with the density of air. Chlorine gas weighs 0.187 lbs/ft^3. (Standard density of air = 0.075 lb/ft^3)

ANSWERS:

1. 2.6
2. 0.9
3. 1.04
4. 1.33
5. 1.26
6. 1.48
7. 57.4 lbs/ft^3
8. 9.5 lbs/gal
9. 12.76 lbs/gal
10. 95 lbs/ft^3
11. 9,617.7 lbs
12. 360,481 lbs/day
13. a. 191.8 lbs/min
    b. 221 lbs/min
14. a. 168,134 lbs/day
    b. 163,296 lbs/day
15. 2.49

Specific Gravity

Sample	Sample Size	Sample Weight	Specific Gravity (using balance)	Specific Gravity (using hydrometer)

$$\text{Specific Gravity} = \frac{\text{weight of 100 mL of solution, g}}{100 \text{ g}}$$

Potassium Permanganate

California State University: Sacramento

Oxidative Processes

- Oxidation – destructive technique used to control tastes and odors by chemical application
- Objectionable compounds broken down into less objectionable by-products
- Common chemical oxidants
  - Chlorine
  - Potassium permanganate/sodium permanganate
  - Ozone
  - Chlorine dioxide

Potassium Permanganate ($KMnO_4$)

- Used to oxidize natural organic matter (NOM), iron, manganese, and sulfide compounds
- Produces intense purple color initially
- Changes from purple to yellow/brown during reactions with compounds being oxidized
  - Manganese dioxide (MnO_2) – insoluble precipitate
  - If any permanganate remains in solution, pink water will pass through filters and go to customer

Potassium Permanganate ($KMnO_4$)

- To minimize finished pink water
  - Measure time required for pink to disappear in jar test
  - Visually observe plant process to determine when pink disappears
    - Each operator must use the same visual cues
- Feeding at intake allows for higher feed rates and more contact time
- Permanganate overdose
  - Feed powdered activated carbon (PAC)
  - Increase pH to increase manganese precipitation rate (extreme measure)

Dosing of Permanganate

- Determining dosage should be determined in the lab with a jar test
  - Run the jar test for an amount of time and RPMs appropriate for your plant
    - Jar test should mimic the plant process
  - Add enough permanganate to maintain a residual
  - Sample jar test water every 30 minutes until contact time is reached
    - Subtract ending residual from initial dosage to determine demand

Dose – residual = demand

Dosing of Permanganate

- 1 mg/L of $KMnO_4$ required to oxidize 0.92 mg/L of soluble iron
- 1 mg/L of $KMnO_4$ required to oxidize 1.92 mg/L of soluble manganese
- If demand is found to be higher than the current residual in the flash mix, increase dosage and run a new demand test
- If demand is going down, decrease feed rate to prevent an overdosage and run a new demand test
  - Overdosing can lead to membrane filter fouling, manganese dioxide in the clearwell and distribution system, as well as pink water in customers' homes

Testing for Permanganate Demand

- Permanganate residual is determined in the same manner as chlorine residual
  - Colorimetric
    - Follow method for reagent used
      - i.e. free chlorine DPD or total chlorine DPD
  - Amperometric titration
- If permanganate concentration is less than 1 mg/L, multiply reading by the concentration
  - Example: if using $KMnO_4$ stock that is 0.891 g/L, multiply residual by 0.891 to get actual mg/L residual

Permanganate Residual

Potassium permanganate ($KMnO_4$) is used in water treatment for the oxidation of iron, manganese, and substances causing taste and odor. It has also been used to control zebra mussels. Potassium permanganate is a dark purple crystalline solid that produces a solution ranging from faint pink (dilute) to deep purple (concentrated).

As permanganate oxidizes compounds in the water, it changes from purple to brown. The final product is manganese dioxide (MnO_2), an insoluble precipitate that can be removed by sedimentation or filtration. All of the $KMnO_4$ must be converted to MnO_2 prior to filtration. Otherwise the pink color may pass through the filters and into the clearwell. On the other hand, if too little permanganate is added, dissolved iron and manganese may pass through the treatment process and into the clearwell. Once chlorine is added, these minerals may then precipitate out, creating color problems in the distribution system.

Measuring permanganate residual at different stages of the treatment process may be helpful in ensuring that the proper dose is being added. The jar test may also be used in determining the best dosage.

Solutions of potassium permanganate for use in the lab may be stored for a long time if they are made with oxidant-demand-free water and kept in an amber bottle out of sunlight.

Sampling and Storage

Samples collected from source waters with an oxidant demand should be analyzed immediately.

- Procedure for measuring free chlorine using DPD Colorimetric Method
  - Procedure
    - Follow the instructions for measuring chlorine residual. Multiply the reading in mg/L by the conversion factor of 0.891 to convert the chlorine reading to permanganate.
    - Page 273 in *Hach Water Analysis Handbook* for chlorine residual method
  - Reagents
    - DPD Powder Pillows for Free Chlorine
  - Apparatus
    - Spectrophotometer
    - Hach Pocket Cl_2 Colorimeter

- Procedure for measuring free chlorine using Amperometric Titration Method
  - Follow manufacturer's instructions

CHLORINE RESIDUAL

Water Treatment Lab Week 1

Chlorination

- **Chlorination** is the process of adding chlorine to water for the destruction or inactivation of pathogenic (disease-causing) organisms
- Chlorine residual is measured in mg/L Cl_2

Chemistry of Chlorination

$$\underset{\text{chlorine gas (liquid chlorine)}}{Cl_2} + H_2O \rightarrow \underset{\text{hypochlorous acid}}{HOCl} + \underset{\text{hydrochloric acid}}{HCl}$$

- Chlorine gas added to water reacts to form hypochlorous acid and hydrochloric acid
- Hypochlorous acid is the disinfecting agent
  - More concentrated at pH > 7
- Hydrochloric acid will cause an increase in the water's pH

Chemistry of Chlorination

- Hypochlorous Acid
  - Dissociates at higher pH:

$$\underset{\text{hypochlorous acid}}{HOCl} \rightarrow \underset{\text{hydrogen ion}}{H^+} + \underset{\text{hypochlorite ion}}{OCl^-}$$

- Hypochlorite ion is only 1% as effective as hypochlorous acid

	Form of free chlorine residual
@ pH less than 7.0	all HOCl
@ pH = 7.5	½ HOCl ; ½ OCl^-
@ pH > 8.5	all OCl^-

- Therefore, as pH ↑ the HOCl concentration will ↓ , meaning that disinfection efficiency will decrease.

Chemistry of Hypochlorination

$$\underset{\text{sodium hypochlorite (bleach)}}{NaOCl} + H_2O \rightarrow \underset{\text{hypochlorous acid}}{HOCl} + \underset{\text{sodium hydroxide (caustic)}}{NaOH}$$

- The same disinfecting agent results (HOCl) despite using a different chemical.
  - Will dissociate at pH > 7.0
- Sodium hypochlorite will slightly raise the pH because of the sodium hydroxide (NaOH) added to the water as a by-product.

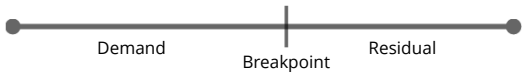
CHLORINE RESIDUALS

Reducing Agents

- Reducing agent – any substance that will readily give up electrons
  - *Any substance in the water that will* reduce *the concentration of the free chlorine.*
- Organic matter
- Inorganic matter
  - Iron
  - Manganese
  - Nitrites
  - Sulfur
  - Ammonia
- Bacterial contamination
- No free chlorine residual will be formed until ALL reducing agents are destroyed.

Breakpoint Chlorination

- The process of adding chlorine to water until the chlorine demand has been satisfied
  - Further additions of chlorine will result in a chlorine residual that is directly proportional to the amount of chlorine added beyond the breakpoint



Dose = Demand + Residual

Chlorination Terminology

- **Chlorine Demand** – the amount of chlorine required to destroy all reducing agents
- **Breakpoint** – the point at which the chlorine dosage has met the demand; any additional chlorine will result in a free residual
- **Chlorine Residual** – The concentration of chlorine present in water after the chlorine demand has been satisfied.
  - Also called residual chlorine
  - The concentration is expressed in terms of the total chlorine residual, which includes both the free and combined or chemically bound chlorine residuals

Free Chlorine Residuals

- **Free available chlorine residual** – That portion of the total available chlorine residual composed of dissolved chlorine gas (Cl_2), hypochlorous acid (HOCl), or hypochlorite ion (OCl^-) remaining in the water after chlorination at the end of a specified contact period.
  - This does not include chlorine that has combined with ammonia, nitrogen, or other compounds.
- **Free residual chlorination** – The application of chlorine to water to produce a free available chlorine residual equal to at least 80% of the total chlorine residual.

Combined Chlorine Residuals

- **Combined available chlorine residual** – the concentration of chlorine residual that is combined with ammonia, organic nitrogen, or both in water as a chloramine and yet is still available to oxidize organic matter and help kill bacteria.
- **Combined chlorine** – the sum of the chlorine species composed of free chlorine and ammonia, including monochloramine, dichloramine, and trichloramine (nitrogen trichloride).

Total Chlorine Residuals

- **Residual chlorine** - The concentration of chlorine present in water after the demand has been satisfied.
  - Also called chlorine residual.
- **Total chlorine** – The total concentration of chlorine in water, including the combined chlorine and the free available chlorine.
- **Total chlorine residual** – The total amount of chlorine (including both free chlorine and chemically bound chlorine) residual present in a water sample after a given time.

Total Residual Chlorine = Combined Residual + Free Residual

CHLORINE TESTING

Chlorine Demand

- The difference between the chlorine added to the water and the amount of residual chlorine remaining after a given time

Demand = Dose - Residual

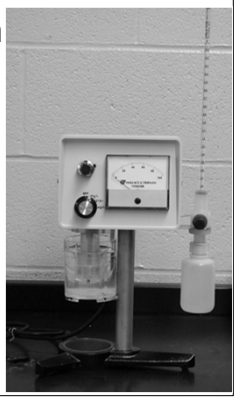
- Chlorine demand can be measured by using separate samples (same source, divided up into separate samples) each dosed with a series of increasing doses of chlorine
- After appropriate contact time, measure free chlorine residual
- Demand is determined by the difference between the original dose and the final free residual concentration

Disinfection Control Tests

- Chlorine Residual
  - Presence of residual
  - Free or combined
  - Concentration
  - Test Methods:
    - Amperometric titration (most accurate) OR
    - Colorimetric (DPD) method
- Bacteriological Test
  - Indicates fecal contamination
  - Coliforms are more resistant to chlorine than other fecal bacteria, if coliforms are not present, other fecals will not be present either

Amperometric Titration

- Accurate and unaffected by sample color or turbidity
- Takes greater skill to use than DPD method with colorimetric devices



Amperometric Titration Procedure

- Fill burette with 0.0056N **phenylarsine oxide (PAO)**
- Measure 200 mL samples into cell and place in the holder on the titrator
- Unless sample pH is known to be between 6.5 and 7.5, add 1 mL of pH 7 phosphate buffer to produce pH of 6.5 – 7.5
- Turn on stirrer and adjust control knob until meter reads max on scale
- Add PAO in 0.1 mL increments
  - This should cause the meter reading to deflect downward
- Adjust the control knob as needed to keep the pointer on the scale
- End-point is reached when the addition of titrant no longer results in a downward deflection
- Read the burette
  - Subtracting the last amount added (that did not cause a downward deflection)
  - The burette reading in mL equals the free chlorine residual in mg/L

Sampling and Collection – SM4500

- Chlorine in aqueous solution is not stable, and the chlorine content of samples or solutions, particularly weak solutions, will decrease rapidly.
- Exposure to sunlight or other strong light or agitation will accelerate the reduction of chlorine.
- Therefore, start chlorine determinations immediately after sampling, avoiding excessive light and agitation.
- Do not store samples to be analyzed for chlorine.

Calibration vs Verification

- Calibration colorimetric equipment with **primary** (liquid) standards of chlorine or potassium permanganate
- Colorimetric equipment verification is performed with **secondary** (gel) standards

Method Update Rule
For Backwash Lagoons that have Discharge Permits
May 10, 2013
Updated December 2018
Fleming Training Center

40CFR136

40 CFR 136 05-21-2012 Table 1B

Parameter	Methodology	EPA	Standard Methods	ASTM	USGS/AOAC/ Other
Chlorine-Total Residual, mg/L	Amperometric Direct	……	4500-Cl D-2000	D1253-08	
	Amperometric Direct (Low Level)	……	4500-Cl E-2000		
	Iodometric Direct	……	4500-Cl B-2000		
	Back Titration Ether End-Point[15]	……	4500-Cl C-2000		
	DPD-FAS	……	4500-Cl F-2000		
	Spectrophotometric, DPD	……	**4500-Cl G-2000**		
	Electrode	22.	TDEC - Fleming Training Center	……	See Footnote [16]

12 Quality Control Elements

1. DOC – demonstration of capability
2. MDL – method detection level
3. LRB/MB – method blank
4. LFB – laboratory fortified blank (standard)
5. LFM/LFMD – laboratory fortified matrix/duplicate (spike)
6. Internal standards, surrogate standards or tracer – ***only applies to organic analysis and radiochemistry***
7. Calibration- initial and continuing
8. Control charts or other trend analysis
9. Corrective action – root cause analysis
10. QC acceptance criteria
11. Definition of a batch (preparation and analytical)
12. Minimum frequency for conducting all QC elements
13. Unwritten 13th Step – SOP – Standard Operating Procedures need to be written and followed for all lab sampling and analyses

Not all of these items apply to all tests, there are many exceptions!

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- DOC
- MDL
- LRB
- LFB
- Dup
- ICAL/CCV
- Control Charts
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Demonstration of Capability (DOC)
  - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
    - No limits listed for chlorine
  - Real people language: each operator running this test need to analyze 4 samples of a Chlorine Standard or Potassium Permanganate ($KMnO_4$) at a concentration around 0.5 mg/L.
  - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
  - Recommend backup analyst do this once a year.

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Method Detection Limit
  - HACH- Estimated Detection Level=0.02mg/L
  - Initial MDL From SM 1030 C.
    - **Make a 0.10 mg/L standard**
    - **Analyze 7 portions over ≥ 3 days**
    - Calculate standard deviation (s)
    - s***** 3.14= MDL
  - Ongoing MDL data
    - Run two (2) spiked samples at least every quarter
    - Record results of method results for each discharge testing

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Laboratory Reagent Blank
  - Real people language: analyze distilled water as a sample by adding DPD powder pillow and waiting the 3-6 minutes before reading
    - Target value is less than MDL
    - Run **Daily (or day of for monthly reporting purposes)**
- Laboratory Fortified Blank
  - Real people language: analyze a chlorine standard or potassium permanganate ($KMnO_4$) at a concentration around 0.5 mg/L
    - Run on a 5% basis, one for every 20 samples **(or day of for monthly reporting purposes)**
- Duplicates of the sample
  - Run on a 5% basis, one for every 20 samples **(or day of for monthly reporting purposes)**
  - Calculate %RPD, ≤ 20%

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Initial Calibration
  - Prepare a set of chlorine standard or potassium permanganate ($KMnO_4$) in accordance with the Guidance for Secondary Standards Use in Calibration **monthly**.
    - Once per month at minimum, before the use of new DPD reagents, or the use of new gel standards

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Stock Standard Solution
  - 0.891 grams of reagent grade $KMnO_4$ in 1000 mL vol. flask made to mark with deionized water.
  - Deionized water must never be stored in plastic containers or exposed to airborne contamination.
  - Store the stock solution in amber bottle in a cool area.
  - The typical shelf life of the stock solution is six (6) months.
  - If solids appear in the solution, **do not use.**

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Intermediate (Working) Standard Solution
  - 10 mL of STOCK made in 1000 mL vol. flask made to mark with deionized water.
  - The flask should be labeled with the name, $KMnO_4$, date of preparation, initials of who made it.
  - This information should also be entered into a logbook.
- ******The intermediate stock solution should be stable for approximately 5 days if kept cool and away from light.******

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Potassium Permanganate Standard Solution
  - Care should be taken that the pipette and glassware are clean and thoroughly rinsed with deionized water to avoid contamination.
  - Store only in glass container (preferably amber glass) never in plastic containers.
  - The working solution should be remade if solids appear in the bottom of the container.

Total Residual Chlorine SM4500-Cl G - 2000, DPD

* Calibration Standard Solutions
  * Four to five calibration standard solutions should be made according to the table below to create a calibration curve once per month at a minimum.
  * The linear regression of the curve should correlate to 0.995 or better.
  * This curve is then used to check or calibrate the instrument.
  * Gel standards are run against the curve and must agree to within + 10%.
* **The working solution should be stable for approximately 2 hours and will fade rapidly (within 15 minutes) if chlorine demand-free water is not used.**

Total Residual Chlorine SM4500-Cl G - 2000, DPD

* Calibration Standard Solutions
  * A target value (e.g. permit value for a facility) should be known and three gel standards, 0.00 mg/L, blank, and two other standards (a low and a high standard) that bracket the target value should be chosen.
  * Gel standards are run against the curve and must agree to within + 10%.

Total Residual Chlorine SM4500-Cl G - 2000, DPD

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
20 mL (vol. Pipette) to 100 mL (vol. flask)	2.0 mg/L
10 mL (vol. Pipette) to 100 mL (vol. flask)	1.0 mg/L
5 mL (vol. Pipette) to 100 mL (vol. flask)	0.5 mg/L
1 mL (vol. Pipette) to 100 mL (vol. flask)	0.1 mg/L
1 mL (vol. Pipette) to 200 mL (vol. flask)	0.05 mg/L
1 mL (vol. Pipette) to 500 mL (vol. flask)	0.02 mg/L
100 mL of deionized water	0.00 mg/L

Don't forget to use DPD on Potassium Permanganate standards

Total Residual Chlorine SM4500-Cl G - 2000, DPD

* Calibration Verification **daily (or day of for monthly reporting purposes)**
* Verify meter with secondary gel standards using a minimum of one blank and two gel standards that bracket the expected sample concentration
* OR verify meter after running samples with a standard of chlorine or potassium permanganate

Chlorine Lab Math

1. A water system has a chlorine demand of 4.1 mg/L and wants to have a 1.1 mg/L residual. What would be the dose?

2. A system just had a main break. The chlorine level of 3.3 mg/L has dropped to 0.3 mg/L. What is the chlorine demand?

3. A water plant treats 7.5 MGD. If the chlorine dose needs to be 3 mg/L, what is the chlorine requirement in pounds per day?

4. Determine the chlorine dose in mg/L if 13 pounds of chlorine are fed while treating 968,000 gallons of water.

5. How many pounds of 65% available chlorine HTH is needed to make 1 gallon of 10% solution?

6. How many gallons of bleach (15% available chlorine) will it take to make a 4% solution when added to enough water to make 50 gallons of hypochlorite?

Answers:
1. 5.2 mg/L
2. 3.0 mg/L
3. 188 lbs/day
4. 1.62 mg/L
5. 1.28 lbs
6. 13 gallons

Chlorine

Calibration Curve & Method Detection Limit (MDL)

The Use of Secondary Standards for Spectrophotometer/Colorimeter Calibration

- Secondary standards (gel standards) are specifically designed to verify the instrument's calibration and to check the instrument's performance.
- They are not intended to be used to create calibration curves or to calibrate the instrument.
- Because the DPD reagent cannot be mixed with the gel standards, the quality and the reaction time of the reagent cannot be assessed.
- For these reasons gel standards cannot take the place of primary standards.

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The Use of Secondary Standards for Spectrophotometer/Colorimeter Calibration

- The analyst is responsible for the following:
  - Preparing the calibration curve for each instrument once per month at a minimum with chlorine standards or potassium permanganate (see instructions below for $KMnO_4$), before the use of new DPD reagents, or the use of new gel standards
  - Recording reagent lot #'s for reagents and standards
  - Recording calibration concentrations
  - Verifying the calibration curve using a minimum of one blank and two gel standards that bracket the expected sample concentration
  - Recording all verification data

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Potassium permanganate ($KMnO_4$) stock standard solution

- 0.891 grams of reagent grade $KMnO_4$ in 1000 mL vol. flask made to mark with deionized water.
- Deionized water must never be stored in plastic containers or exposed to airborne contamination. Store the stock solution in an amber bottle in a cool area.
- The typical shelf life of the stock solution is six (6) months. If solids appear in the solution, do not use
- ***Avoid leaving the cap off for extended periods of time and avoid contamination.***

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Intermediate (working) standard solution (10 mg/L)

- 10 mL of STOCK made in 1000 mL vol. flask made to mark with deionized water.
- The flask should be labeled with the name, $KMnO_4$, date of preparation, and initials of who made it.
- This information should also be entered into a logbook
- **The intermediate stock solution should be stable for approximately 5 days if kept cool and away from light.*
- Care should be taken that the pipette and glassware are clean and thoroughly rinsed with deionized water to avoid contamination.
- Store only in a glass container (preferably amber glass) never in plastic containers. The working solution should be remade if solids appear in the bottom of the container

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Calibration Standard Solutions

- If using $KMnO_4$, four to five calibration standard solutions should be made according to the table on the next slide with the addition of DPD to create a calibration curve ***once per month*** at a minimum.
- The correlation coefficient of the curve should correlate to 0.995 or better.
- This curve is then used to check instrument calibration.
- Gel standards are run against the curve and must agree to within $\pm$ 10%
- **The working solution should be stable for approximately 2 hours and will fade rapidly (within 15 minutes) if chlorine demand-free water is not used.**

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ICV/CCV –Chlorine

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
20 mL (vol. Pipette) to 100 mL (vol. flask)	2.0 mg/L
10 mL (vol. Pipette) to 100 mL (vol. flask)	1.0 mg/L
5 mL (vol. Pipette) to 100 mL (vol. flask)	0.5 mg/L
1 mL (vol. Pipette) to 100 mL (vol. flask)	0.1 mg/L
½ mL (vol. Pipette) to 100 mL (vol. flask)	0.05 mg/L
1 mL (vol. Pipette) to 500 mL (vol. flask)	0.02 mg/L
100 mL of deionized water	0.00 mg/L

Don't forget to use DPD on Potassium Permanganate standards

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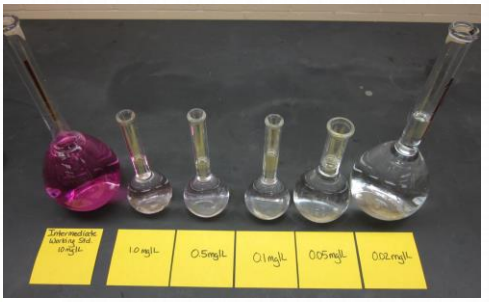
Calibration Standard Solutions



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Dilutions for Today's Example – Pick 4

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
20 mL (vol. Pipette) to 100 mL (vol. flask)	2.0 mg/L
10 mL (vol. Pipette) to 100 mL (vol. flask)	1.0 mg/L
5 mL (vol. Pipette) to 100 mL (vol. flask)	0.5 mg/L
1 mL (vol. Pipette) to 100 mL (vol. flask)	0.1 mg/L
½ mL (vol. Pipette) to 100 mL (vol. flask)	0.05 mg/L
1 mL (vol. Pipette) to 500 mL (vol. flask)	0.02 mg/L

Don't forget to use DPD on Potassium Permanganate standards

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Subject	true value (x)	value read (y)
1	0.02	0.02
2	0.05	0.03
3	0.10	0.05
4	0.50	0.42
5	1.00	0.91
R^2 =	0.997567397	

Although, still greater than 0.995

Not a good recovery, tried a second dilution

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Calibration Standard Solutions

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Subject	true value (x)	value read (y)
1	0.02	0.02
2	0.05	0.03
3	0.10	0.09
4	0.50	0.42
5	1.00	0.91
R^2 =	0.99833	

Better than previous calibration curve

Better recovery with second dilution

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Method Detection Limit (MDL)

- 1020 B. 4 – As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level (5 x 0.010 mg/L = 0.050 mg/L).
  - Ideally, prepare and analyze at least seven (7) portions of this solution over a 3-day period to ensure that the MDL determination is more representative of routine measurements as performed in the laboratory.
  - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD (relative standard deviation) values ≤ 20%.
- 4020 B.1.b. – Verify MDL at least annually.
  - Ideally use pooled data from several analysts rather than data from one analyst.

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Method Detection Limit (MDL)

- Real people language – have several operators, who run this test, analyze chlorine or Potassium Permanganate ($KMnO_4$) standards at a concentration of 0.05 mg/L over several days with a total of at least 7 samples
  - Joe analyzes 3 samples on Monday
  - Bob analyzes 3 samples on Tuesday
  - Mary analyzes 3 samples on Wednesday
- Run this once a year

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Method Detection Limit (MDL)

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Dilutions for Today's Example for MDL

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
½ mL (vol. Pipette) to 100 mL (vol. flask)	0.05 mg/L

Don't forget to use DPD on Potassium Permanganate standards

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Method Detection Limit (MDL_s)

Date	Analyst	Number	True Value	Value Read	% Recovery (50-150%)
4/12/2018	BGL	1	0.05	0.05	93.33
4/12/2018	BGL	2	0.05	0.05	96.67
4/13/2018	BGL	3	0.05	0.05	66.67
4/13/2018	BGL	4	0.05	0.05	66.67
4/14/2018	BGL	5	0.05	0.05	100.00
4/14/2018	BGL	6	0.05	0.05	83.33
4/14/2018	BGL	7	0.05	0.05	90.00

Spike Standard Dev	0.01272
Spike Average	0.05571
Data Points <24 mo.	7
Students t value	3.14267
Required # of Dates	PASS
Required # of Replicates	PASS
Spike Level Evaluation	PASS
Spiked MDL	0.0400
Blank MDL	0.0300
Minimum Level	0.1200
Method Detection Limit	0.0400

Enter a date in cell I3 below. MDLs will be re-evaluated based on the most recent 24 months' data.

04/16/2018

Enter Analyte Name

Total Chlorine

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Method Detection Limit (MDL_b)

Date	Analyst	Number	Method Blank
4/12/2018	BGL	1	0.01
4/12/2018	BGL	2	0.01
4/13/2018	BGL	3	0.02
4/13/2018	BGL	4	0.03
4/14/2018	BGL	5	0.02
4/14/2018	BGL	6	0
4/15/2018	BGL	7	nd

Analyte Name

Total Chlorine

Blank Standard Deviation	0.01049
Blank Average	0.01500
Data Points <24 mo.	7
Students t value	3.14267
Required # of Dates	PASS
Required # of Blanks	PASS
Blank MDL	0.0300

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4500-Cl CHLORINE (RESIDUAL)*

4500-Cl A. Introduction

1. Effects of Chlorination

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulfide, and some organic substances.

Chlorination may produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloroorganic compounds, such as chloroform, may be formed. Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life. To fulfill the primary purpose of chlorination and to minimize any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

2. Chlorine Forms and Reactions

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. The relative proportion of these free chlorine forms is pH- and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.

Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride. The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand, and reaction time. Both free and combined chlorine may be present simultaneously. Combined chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts. Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically, the principal analytical problem has been to distinguish between free and combined forms of chlorine.

3. Selection of Method

In two separate but related studies, samples were prepared and distributed to participating laboratories to evaluate chlorine methods. Because of poor accuracy and precision and a high overall (average) total error in these studies, all orthotolidine procedures except one were dropped in the 14th Edition of this work. The useful stabilized neutral orthotolidine method was deleted from the 15th Edition because of the toxic nature of orthotolidine. The leuco crystal violet (LCV) procedure was dropped from the 17th Edition because of its relative difficulty and the lack of comparative advantages.

a. Natural and treated waters: The iodometric methods (4500-Cl.B and C) are suitable for measuring total chlorine concentrations greater than 1 mg/L, but the amperometric endpoint of 4500-Cl.C and D gives greater sensitivity. All acidic iodometric methods suffer from interferences, generally in proportion to the quantity of potassium iodide (KI) and H^+ added.

The amperometric titration method (4500-Cl.D) is a standard of comparison for the determination of free or combined chlorine. It is affected little by common oxidizing agents, temperature variations, turbidity, and color. The method is not as simple as the colorimetric methods and requires greater operator skill to obtain the best reliability. Loss of chlorine can occur because of rapid stirring in some commercial equipment. Clean and conditioned electrodes are necessary for sharp endpoints.

A low-level amperometric titration procedure (4500-Cl.E) has been added to determine total chlorine at levels below 0.2 mg/L. This method is recommended only when quantification of such low residuals is necessary. The interferences are similar to those found with the standard amperometric procedure (4500-Cl.D). The DPD methods (4500-Cl.F and G) are operationally simpler for determining free chlorine than the amperometric titration. Procedures are given for estimating the separate mono- and dichloramine and combined fractions. High concentrations of monochloramine interfere with the free chlorine determination unless the reaction is stopped with arsenite or thioacetamide. In addition, the DPD methods are subject to interference by oxidized forms of manganese unless compensated for by a blank.

The amperometric and DPD methods are unaffected by dichloramine concentrations in the range of 0 to 9 mg Cl as Cl_2/L in the determination of free chlorine. Nitrogen trichloride, if present, may react partially as free chlorine in the amperometric, DPD, and FACTS methods. The extent of this interference in the DPD methods does not appear to be significant.

The free chlorine test, syringaldazine (FACTS, 4500-Cl.H) was developed specifically for free chlorine. It is unaffected by significant concentrations of monochloramine, dichloramine, nitrate, nitrite, and oxidized forms of manganese.[1]

Sample color and turbidity may interfere in all colorimetric procedures.

Organic contaminants may produce a false free chlorine reading in most colorimetric methods (see ¶ *b* below). Many strong oxidizing agents interfere in the measurement of free chlorine in all methods. Such interferences include bromine, chlorine dioxide, iodine, permanganate, hydrogen peroxide, and ozone. However, the reduced forms of these compounds—bromide, chloride, iodide, manganous ion, and oxygen, in the absence of other oxidants, do not interfere. Reducing agents, such as ferrous compounds, hydrogen sulfide, and oxidizable organic matter, generally do not interfere.

* Approved by Standard Methods Committee, 2000. Editoral revisions, 2011.

https://doi.org/10.2105/SMWW.2882.078

b. Wastewaters: The determination of total chlorine in samples containing organic matter presents special problems. Because of the presence of ammonia, amines, and organic compounds, particularly organic nitrogen, residual chlorine exists in a combined state. A considerable residual may exist in this form, but at the same time there may be appreciable unsatisfied chlorine demand. Addition of reagents in the determination may change these relationships so residual chlorine is lost during the analysis. Only the DPD method for total chlorine is performed under neutral pH conditions. In wastewater, the differentiation between free chlorine and combined chlorine ordinarily is not made because wastewater chlorination seldom is carried far enough to produce free chlorine.

The determination of residual chlorine in industrial wastes is similar to that in domestic wastewater when the waste contains organic matter, but may be similar to the determination in water when the waste is low in organic matter.

None of these methods is applicable to estuarine or marine waters because the bromide is converted to bromine and bromamines, which are detected as free or total chlorine. A procedure for estimating this interference is available for the DPD method.

Although the methods given below are useful for the determination of residual chlorine in wastewaters and treated effluents, select the method in accordance with sample composition. Some industrial wastes, or mixtures of wastes with domestic wastewater, may require special precautions and modifications to obtain satisfactory results.

Determine free chlorine in wastewater by any of the methods provided that known interfering substances are absent or appropriate correction techniques are used. The amperometric method is the method of choice because it is not subject to interference from color, turbidity, iron, manganese, or nitrite nitrogen. The DPD method is subject to interference from high concentrations of monochloramine, which is avoided by adding thioacetamide immediately after reagent addition. Oxidized forms of manganese at all levels encountered in water will interfere in all methods except in the free chlorine measurement of amperometric titrations and FACTS, but a blank correction for manganese can be made in 4500-Cl.F and G.

The FACTS method is unaffected by concentrations of monochloramine, dichloramine, nitrite, iron, manganese, and other interfering compounds normally found in domestic wastewaters.

For total chlorine in samples containing significant amounts of organic matter, use either the DPD methods (4500-Cl.F and G), amperometric, or iodometric back titration method (4500-Cl.C) to prevent contact between the full concentration of liberated iodine and the sample. With 4500-Cl.C, do not use the starch-iodide endpoint if the concentration is less than 1 mg/L. In the absence of interference, the amperometric and starch-iodide endpoints give concordant results. The amperometric endpoint is inherently more sensitive and is free of interference from color and turbidity, which can cause difficulty with the starch-iodide endpoint. On the other hand, certain metals, surface-active agents, and complex anions in some industrial wastes interfere in the amperometric titration and indicate the need for another method for such wastewaters. Silver in the form of soluble silver cyanide complex, in concentrations of 1.0 mg Ag/L, poisons the cell at pH 4.0 but not at 7.0. The silver ion, in the absence of the cyanide complex, gives extensive response in the current at pH 4.0 and gradually poisons the cell at all pH levels. Cuprous copper in the soluble copper cyanide ion, in concentrations of 5 mg Cu/L or less, poisons the cell at pH 4.0 and 7.0. Although iron and nitrite may interfere with this method, minimize the interference by buffering to pH 4.0 before adding KI. Oxidized forms of manganese interfere in all methods for total chlorine including amperometric titration. An unusually high content of organic matter may cause uncertainty in the endpoint.

Regardless of endpoint detection, either phenylarsine oxide or thiosulfate may be used as the standard reducing reagent at pH 4. The former is more stable and is preferred.

The DPD titrimetric and colorimetric methods (4500-Cl.F and G, respectively) are applicable to determining total chlorine in polluted waters. In addition, both DPD procedures and the amperometric titration method allow for estimating monochloramine and dichloramine fractions. Because all methods for total chlorine depend on the stoichiometric production of iodine, waters containing iodine-reducing substances may not be analyzed accurately by these methods, especially where iodine remains in the solution for a significant time. This problem occurs in 4500-Cl.B and D. The back titration procedure (4500-Cl.C) and 4500-Cl.F and G cause immediate reaction of the iodine generated so it has little chance to react with other iodine-reducing substances.

In all colorimetric procedures, compensate for color and turbidity by using color and turbidity blanks.

A method (4500-Cl.I) for total residual chlorine using a potentiometric iodide electrode is proposed. This method is suitable for analysis of chlorine residuals in natural and treated waters and wastewater effluents. No differentiation of free and combined chlorine is possible. This procedure is an adaptation of other iodometric techniques and is subject to the same inferences.

4. Sampling and Storage

Chlorine in aqueous solution is not stable, and the chlorine content of samples or solutions, particularly weak solutions, will decrease rapidly. Exposure to sunlight or other strong light or agitation will accelerate the reduction of chlorine. Therefore, start chlorine determinations immediately after sampling, avoiding excessive light and agitation. Do not store samples to be analyzed for chlorine.

5. Reference

1. Cooper, W.J., N.M. Roscher & R.A. Slifer. 1982. Determining free available chlorine by DPD-colorimetric, DPD-steadifac (colorimetric) and FACTS procedures. *J. Amer. Water Works Assoc.* 74:362.

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https://doi.org/10.2105/SMWW.2882.078

4500-Cl B. Iodometric Method I

1. General Discussion

a. Principle: Chlorine will liberate free iodine from potassium iodide (KI) solutions at pH 8 or less. The liberated iodine is titrated with a standard solution of sodium thiosulfate ($Na_2S_2O_3$) with starch as the indicator. Titrate at pH 3 to 4 because the reaction is not stoichiometric at neutral pH due to partial oxidation of thiosulfate to sulfate.

b. Interference: Oxidized forms of manganese and other oxidizing agents interfere. Reducing agents, such as organic sulfides, also interfere. Although the neutral titration minimizes the interfering effect of ferric and nitrite ions, the acid titration is preferred because some forms of combined chlorine do not react at pH 7. Use only acetic acid for the acid titration; sulfuric acid (H_2SO_4) will increase interferences; never use hydrochloric acid (HCl). See 4500-Cl.A.3 for discussion of other interferences.

c. Minimum detectable concentration: The minimum detectable concentration approximates 40 μg Cl as Cl_2/L if 0.01*N* $Na_2S_2O_3$ is used with a 1000-mL sample. Concentrations below 1 mg/L cannot be determined accurately by the starch-iodide endpoint used in this method. Lower concentrations can be measured with the amperometric endpoint in 4500-Cl.C and D.

d. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:I.

2. Reagents

a. Acetic acid, conc (glacial).

b. Potassium iodide (KI), crystals.

c. Standard sodium thiosulfate, 0.1*N:* Dissolve 25 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 L freshly boiled distilled water and standardize against potassium bi-iodate or potassium dichromate after at least 2 weeks storage. This initial storage is necessary to allow oxidation of any bisulfite ion present. Use boiled distilled water and add a few milliliters chloroform ($CHCl_3$) to minimize bacterial decomposition.

Standardize 0.1*N* $Na_2S_2O_3$ by one of the following:

1) Iodate method—Dissolve 3.249 g anhydrous potassium bi-iodate, $KH(IO_3)_2$, primary standard quality, or 3.567 g KIO_3 dried at 103 ± 2°C for 1 h, in distilled water and dilute to 1000 mL to yield a 0.1000*N* solution. Store in a glass-stoppered bottle.

   To 80 mL distilled water, add, with constant stirring, 1 mL conc H_2SO_4, 10.00 mL 0.1000*N* $KH(IO_3)_2$, and 1 g KI. Titrate immediately with 0.1*N* $Na_2S_2O_3$ titrant until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch indicator solution and continue titrating until the blue color disappears.

2) Dichromate method—Dissolve 4.904 g anhydrous potassium dichromate, $K_2Cr_2O_7$, of primary standard quality, in distilled water and dilute to 1000 mL to yield a 0.1000*N* solution. Store in a glass-stoppered bottle.

Proceed as in the iodate method, with the following exceptions: Substitute 10.00 mL 0.1000*N* $K_2Cr_2O_7$ for iodate and let reaction mixture stand 6 min in the dark before titrating with 0.1*N* $Na_2S_2O_3$ titrant.

$$\text{Normality } Na_2S_2O_3 = \frac{1}{\text{mL } Na_2S_2O_3 \text{ consumed}}$$

d. Standard sodium thiosulfate titrant, 0.01*N* or 0.025*N:* Improve the stability of 0.01*N* or 0.025*N* $Na_2S_2O_3$ by diluting an aged 0.1*N* solution, made as directed above, with freshly boiled distilled water. Add 4 g sodium borate and 10 mg mercuric iodide/L solution. For accurate work, standardize this solution daily in accordance with the directions given above, using 0.01*N* or 0.025*N* iodate or $K_2Cr_2O_7$. Use sufficient volumes of these standard solutions so their final dilution is not greater than 1 + 4. To speed up operations where many samples must be titrated use an automatic buret of a type in which rubber does not come in contact with the solution. Standard titrants, 0.0100*N* and 0.0250*N*, are equivalent, respectively, to 354.5 μg and 886.3 μg Cl as Cl_2/1.00 mL.

e. Starch indicator solution: To 5 g starch (potato, arrowroot, or soluble), add a little cold water and grind in a mortar to a thin paste. Pour into 1 L of boiling distilled water, stir, and let settle overnight. Use clear supernate. Preserve with 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide/L starch solution. Some commercial starch substitutes are satisfactory.

f. Standard iodine, 0.1*N:* See 4500-Cl.C.3*g*.

g. Dilute standard iodine, 0.0282*N:* See 4500-Cl.C.3*h*.

3. Procedure

a. Volume of sample: Select a sample volume that will require no more than 20 mL 0.01*N* $Na_2S_2O_3$ and no less than 0.2 mL for the starch-iodide endpoint. For a chlorine range of 1 to 10 mg/L, use a 500-mL sample; above 10 mg/L, use proportionately less sample. Use smaller samples and volumes of titrant with the amperometric endpoint.

b. Preparation for titration: Place 5 mL acetic acid, or enough to reduce the pH to between 3.0 and 4.0, in a flask or white porcelain casserole. Add about 1 g KI estimated on a spatula. Pour sample in and mix with a stirring rod.

c. Titration: Titrate away from direct sunlight. Add 0.025*N* or 0.01*N* $Na_2S_2O_3$ from a buret until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch solution and titrate until blue color is discharged.

If the titration is made with 0.025*N* $Na_2S_2O_3$ instead of 0.01*N*, then, with a 1-L sample, 1 drop is equivalent to about 50 μg/L. It is not possible to discern the endpoint with greater accuracy.

d. Blank titration: Correct result of sample titration by determining blank contributed by oxidizing or reducing reagent impurities. The blank also compensates for the concentration of iodine bound to starch at the endpoint.

Take a volume of distilled water corresponding to the sample used for titration in 4500-Cl.B.3*a–c*, add 5 mL acetic acid, 1 g KI, and 1 mL starch solution. Perform blank titration as in ¶s 1) or 2) below, whichever applies.

1) If a blue color develops, titrate with 0.01*N* or 0.025*N* $Na_2S_2O_3$ to disappearance of blue color and record result. *B* (see 4500-Cl.B.4) is negative.

2) If no blue color occurs, titrate with 0.0282*N* iodine solution until a blue color appears. Back-titrate with 0.01*N* or 0.025*N* $Na_2S_2O_3$ and record the difference. *B* is positive.

Before calculating the chlorine concentration, subtract the blank titration of ¶ 1) above from the sample titration; or, if necessary, add the net equivalent value of the blank titration of ¶ 2) above.

4. Calculation

For standardizing chlorine solution for temporary standards:

$$\text{mg Cl as } Cl_2/\text{mL} = \frac{(A \pm B) \times N \times 35.45}{\text{mL sample}}$$

For determining total available residual chlorine in a water sample:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{(A \pm B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,
B = mL titration for blank (positive or negative), and
N = normality of $Na_2S_2O_3$.

5. Precision and Bias

Published studies[1,2] give the results of nine methods used to analyze synthetic water samples without interferences; variations of some of the methods appear in this edition. More current data are not now available.

6. References

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7. Bibliography

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4500-Cl C. Iodometric Method II

1. General Discussion

a. Principle: In this method, used for wastewater analysis, the endpoint signal is reversed because the unreacted standard reducing agent (phenylarsine oxide or thiosulfate) remaining in the sample is titrated with standard iodine or standard iodate, rather than the iodine released being titrated directly. This indirect procedure is necessary regardless of the method of endpoint detection, to avoid contact between the full concentration of liberated iodine and the wastewater.

When iodate is used as a back titrant, use only phosphoric acid. Do not use acetate buffer.

b. Interference: Oxidized forms of manganese and other oxidizing agents give positive interferences. Reducing agents, such as organic sulfides, do not interfere as much as in 4500-Cl.B. Minimize iron and nitrite interference by buffering to pH 4.0 before adding potassium iodide (KI). An unusually high content of organic matter may cause some uncertainty in the endpoint. Whenever manganese, iron, and other interferences definitely are absent, reduce this uncertainty and improve precision by acidifying to pH 1.0. Control interference from more than 0.2 mg nitrite/L with phosphoric acid-sulfamic acid reagent. A larger fraction of organic chloramines will react at lower pH along with interfering substances. See 4500-Cl.A.3 for a discussion of other interferences.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:I.

2. Apparatus

For a description of the amperometric endpoint detection apparatus and a discussion of its use, see 4500-Cl.D.2*a*.

3. Reagents

a. Standard phenylarsine oxide solution, 0.005 64*N:* Dissolve approximately 0.8 g phenylarsine oxide powder in 150 mL 0.3*N* NaOH solution. After settling, decant 110 mL into 800 mL distilled water and mix thoroughly. Bring to pH 6 to 7 with 6*N* HCl and dilute to 950 mL with distilled water. **Caution: Severe poison, cancer suspect agent.**

Standardization—Accurately measure 5 to 10 mL freshly standardized 0.0282*N* iodine solution into a flask and add 1 mL KI solution. Titrate with phenylarsine oxide solution, using the amperometric endpoint (4500-Cl.D) or starch solution (see 4500-Cl.B.2*e*) as an indicator. Adjust to 0.005 64*N* and recheck against the standard iodine solution; 1.00 mL = 200 μg available chlorine. (**Caution: Toxic—take care to avoid ingestion.**)

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b. Standard sodium thiosulfate solution, 0.1*N:* See 4500-Cl.B.2*c*.

c. Standard sodium thiosulfate solution, 0.005 64*N:* Prepare by diluting 0.1*N* $Na_2S_2O_3$. For maximum stability of the dilute solution, prepare by diluting an aged 0.1*N* solution with freshly boiled distilled water (to minimize bacterial action) and add 4 g $Na_4B_4O_7$/L. To inhibit mold formation optionally add either 10 mg HgI_2 or 2 drops toluene per liter of solution. Standardize daily as directed in 4500-Cl.B.2*c* using 0.005 64*N* $K_2Cr_2O_7$ or iodate solution. Use sufficient volume of sample so the final dilution does not exceed 1 + 2. Use an automatic buret of a type in which rubber does not come in contact with the solution. 1.00 mL = 200 μg available chlorine.

d. Potassium iodide (KI), crystals.

e. Acetate buffer solution, pH 4.0: Dissolve 146 g anhydrous $NaC_2H_3O_2$, or 243 g $NaC_2H_3O_2 \cdot 3H_2O$, in 400 mL distilled water, add 480 g conc acetic acid, and dilute to 1 L with chlorine-demand-free water.

f. Standard arsenite solution, 0.1*N:* Accurately weigh a stoppered weighing bottle containing approximately 4.95 g arsenic trioxide, As_2O_3. Transfer without loss to a 1-L volumetric flask and again weigh bottle. Do not attempt to brush out adhering oxide. Moisten As_2O_3 with water and add 15 g NaOH and 100 mL distilled water. Swirl flask contents gently to dissolve. Dilute to 250 mL with distilled water and saturate with CO_2, thus converting all NaOH to $NaHCO_3$. Dilute to mark, stopper, and mix thoroughly. This solution will preserve its titer almost indefinitely. (**Caution: Severe poison. Cancer suspect agent.**)

$$\text{Normality} = \frac{\text{g } As_2O_3}{49.455}$$

g. Standard iodine solution, 0.1*N:* Dissolve 40 g KI in 25 mL chlorine-demand-free water, add 13 g resublimed iodine, and stir until dissolved. Transfer to a 1-L volumetric flask and dilute to mark.

Standardization—Accurately measure 40 to 50 mL 0.1*N* arsenite solution into a flask and titrate with 0.1*N* iodine solution, using starch solution as indicator. To obtain accurate results, ensure that the solution is saturated with CO_2 at end of titration by passing current of CO_2 through solution for a few minutes just before endpoint is reached, or add a few drops of HCl to liberate sufficient CO_2 to saturate solution. Alternatively, standardize against $Na_2S_2O_3$; see 4500-Cl.B.2*c*1).

Optionally, prepare 0.1000*N* iodine solution directly as a standard solution by weighing 12.69 g primary standard resublimed iodine. Because I_2 may be volatilized and lose from both solid and solution, transfer the solid immediately to KI as specified above. Never let solution stand in open containers for extended periods.

h. Standard iodine titrant, 0.0282*N:* Dissolve 25 g KI in a little distilled water in a 1-L volumetric flask, add correct amount of 0.1*N* iodine solution exactly standardized to yield a 0.0282*N* solution, and dilute to 1 L with chlorine-demand-free water. For accurate work, standardize daily according to directions in ¶ *g* above, using 5 to 10 mL of arsenite or $Na_2S_2O_3$ solution. Store in amber bottles or in the dark; protect solution from direct sunlight at all times and keep from all contact with rubber.

i. Starch indicator: See 4500-Cl.B.2*e*.

j. Standard iodate titrant, 0.005 64*N:* Dissolve 201.2 mg primary standard grade KIO_3, dried for 1 h at 103°C, or 183.3 mg primary standard anhydrous potassium bi-iodate in distilled water and dilute to 1 L.

k. Phosphoric acid solution (H_3PO_4), 1 + 9.

l. Phosphoric acid-sulfamic acid solution: Dissolve 20 g NH_2SO_3H in 1 L 1 + 9 phosphoric acid.

m. Chlorine-demand-free water: Prepare chlorine-demand-free water from good-quality distilled or deionized water by adding sufficient chlorine to give 5 mg/L free chlorine. After standing 2 d this solution should contain at least 2 mg/L free chlorine; if not, discard and obtain better-quality water. Remove remaining free chlorine by placing container in sunlight or irradiating with an ultraviolet lamp. After several hours take sample, add KI, and measure total chlorine with a colorimetric method using a nessler tube to increase sensitivity. Do not use before last trace of free and combined chlorine has been removed.

Distilled water commonly contains ammonia and also may contain reducing agents. Collect good-quality distilled or deionized water in a sealed container from which water can be drawn by gravity. To the air inlet of the container add an H_2SO_4 trap consisting of a large test tube half filled with 1 + 1 H_2SO_4 connected in series with a similar but empty test tube. Fit both test tubes with stoppers and inlet tubes terminating near the bottom of the tubes and outlet tubes terminating near the top of the tubes. Connect outlet tube of trap containing H_2SO_4 to the distilled water container, connect inlet tube to outlet of empty test tube. The empty test tube will prevent discharge to the atmosphere of H_2SO_4 due to temperature-induced pressure changes. Stored in such a container, chlorine-demand-free water is stable for several weeks unless bacterial growth occurs.

4. Procedure

a. Preparation for titration:

1) Volume of sample—For chlorine concentration of 10 mg/L or less, titrate 200 mL. For greater chlorine concentrations, use proportionately less sample and dilute to 200 mL with chlorine-demand-free water. Use a sample of such size that not more than 10 mL phenylarsine oxide solution is required.

2) Preparation for titration—Measure 5 mL 0.005 64*N* phenylarsine oxide or thiosulfate for chlorine concentrations from 2 to 5 mg/L, and 10 mL for concentrations of 5 to 10 mg/L, into a flask or casserole for titration with standard iodine or iodate. Start stirring. For titration by amperometry or standard iodine, also add excess KI (approximately 1 g) and 4 mL acetate buffer solution or enough to reduce the pH to between 3.5 and 4.2.

b. Titration: Use one of the following:

1) Amperometric titration—Add 0.0282*N* iodine titrant in small increments from a 1-mL buret or pipet. Observe meter needle response as iodine is added: the pointer remains practically stationary until the endpoint is approached, whereupon each iodine increment causes a temporary deflection of the microammeter, with the pointer dropping back to its original position. Stop titration at endpoint when a small increment of iodine titrant gives a definite pointer deflection upscale and the pointer does not return promptly to its original position. Record volume of iodine titrant used to reach endpoint.

2) Colorimetric (iodine) titration—Add 1 mL starch solution and titrate with 0.0282*N* iodine to the first appearance of blue color that persists after complete mixing.

3) Colorimetric (iodate) titration—To suitable flask or casserole add 200 mL chlorine-demand-free water and add, with agitation, the required volume of reductant, an excess of KI (approximately 0.5 g), 2 mL 10% H_3PO_4 solution, and 1 mL starch solution in the order given, and titrate immediately* with 0.005 64*N* iodate solution to the first appearance of a blue color that persists after complete mixing. Designate volume of iodate solution used as *A*. Repeat procedure, substituting 200 mL sample for the 200 mL chlorine-demand-free water. If sample is colored or turbid, titrate to the first change in color, using for comparison another portion of sample with H_3PO_4 added. Designate this volume of iodate solution as *B*.

5. Calculation

a. Titration with standard iodine:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{(A - 5B) \times 200}{C}$$

where:

A = mL 0.005 64*N* reductant,
B = mL 0.0282 *N* I_2, and
C = mL sample.

b. Titration with standard iodate:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{(A - B) \times 200}{C}$$

where:

A = mL $Na_2S_2O_3$,
B = mL iodate required to titrate $Na_2S_2O_3$, and
C = mL sample.

6. Bibliography

See 4500-Cl.B.7.

* Titration may be delayed up to 10 min without appreciable error if H_3PO_4 is not added until immediately before titration.

4500-Cl D. Amperometric Titration Method

1. General Discussion

Amperometric titration requires a higher degree of skill and care than the colorimetric methods. Chlorine residuals over 2 mg/L are measured best by means of smaller samples or by dilution with water that has neither residual chlorine nor a chlorine demand. The method can be used to determine total chlorine and can differentiate between free and combined chlorine. A further differentiation into monochloramine and dichloramine fractions is possible by control of KI concentration and pH.

a. Principle: The amperometric method is a special adaptation of the polarographic principle. Free chlorine is titrated at a pH between 6.5 and 7.5, a range in which the combined chlorine reacts slowly. The combined chlorine, in turn, is titrated in the presence of the proper amount of KI in the pH range 3.5 to 4.5. When free chlorine is determined, the pH must not be greater than 7.5 because the reaction becomes sluggish at higher pH values, nor less than 6.5 because at lower pH values some combined chlorine may react even in the absence of iodide. When combined chlorine is determined, the pH must not be less than 3.5 because of increased interferences at lower pH values, nor greater than 4.5 because the iodide reaction is not quantitative at higher pH values. The tendency of monochloramine to react more readily with iodide than does dichloramine provides a means for further differentiation. The addition of a small amount of KI in the neutral pH range enables estimation of monochloramine content. Lowering the pH into the acid range and increasing the KI concentration allows the separation determination of dichloramine.

Organic chloramines can be measured as free chlorine, monochloramine, or dichloramine, depending on the activity of chlorine in the organic compound.

Phenylarsine oxide is stable even in dilute solution and each mole reacts with two equivalents of halogen. A special amperometric cell is used to detect the endpoint of the residual chlorine-phenylarsine oxide titration. The cell consists of a nonpolarizable reference electrode that is immersed in a salt solution and a readily polarizable noble-metal electrode that is in contact with both the salt solution and the sample being titrated. In some applications, endpoint selectivity is improved by adding +200 mV to the platinum electrode versus silver, silver chloride. Another approach to endpoint detection uses dual platinum electrodes, a mercury cell with voltage divider to impress a potential across the electrodes, and a microammeter. If there is no chlorine residual in the sample, the microammeter reading will be comparatively low because of cell polarization. The greater the residual, the greater the microammeter reading. The meter acts merely as a null-point indicator—that is, the actual meter reading is not important, but rather the relative readings as the titration proceeds. The gradual addition of phenylarsine oxide causes the cell to become more and more polarized because of the decrease in chlorine. The endpoint is recognized when no further decrease in meter reading can be obtained by adding more phenylarsine oxide.

b. Interference: Accurate determinations of free chlorine cannot be made in the presence of nitrogen trichloride, NCl_3, or chlorine dioxide, which titrate partly as free chlorine. When present, NCl_3 can titrate partly as free chlorine and partly as dichloramine, contributing a positive error in both fractions at a rate of approximately 0.1%/min. Some organic chloramines also can be titrated in each step. Monochloramine can intrude into the

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free chlorine fraction and dichloramine can interfere in the monochloramine fraction, especially at high temperatures and prolonged titration times. Free halogens other than chlorine also will titrate as free chlorine. Combined chlorine reacts with iodide ions to produce iodine. When titration for free chlorine follows a combined chlorine titration, which requires addition of KI, erroneous results may occur unless the measuring cell is rinsed thoroughly with distilled water between titrations.

Interference from copper has been noted in samples taken from copper pipe or after heavy copper sulfate treatment of reservoirs, with metallic copper plating out on the electrode. Silver ions also poison the electrode. Interference occurs in some highly colored waters and in waters containing surface-active agents. Very low temperatures slow response of measuring cell and longer time is required for the titration, but precision is not affected. A reduction in reaction rate is caused by pH values above 7.5; overcome this by buffering all samples to pH 7.0 or less. On the other hand, some substances, such as manganese, nitrite, and iron, do not interfere. The violent stirring of some commercial titrators can lower chlorine values by volatilization. When dilution is used for samples containing high chlorine content, take care that the dilution water is free of chlorine and ammonia and possesses no chlorine demand.

See 4500-Cl.A.3 for a discussion of other interferences.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:I.

2. Apparatus

a. Endpoint detection apparatus, consisting of a cell unit connected to a microammeter, with necessary electrical accessories. The cell unit includes a noble-metal electrode of sufficient surface area, a salt bridge to provide an electrical connection without diffusion of electrolyte, and a reference electrode of silver–silver chloride in a saturated sodium chloride solution connected into the circuit by means of the salt bridge. Numerous commercial systems are available.

Keep platinum electrode free of deposits and foreign matter. Vigorous chemical cleaning generally is unnecessary. Occasional mechanical cleaning with a suitable abrasive usually is sufficient. Keep salt bridge in good operating condition; do not allow it to become plugged nor permit appreciable flow of electrolyte through it. Keep solution surrounding reference electrode free of contamination and maintain it at constant composition by ensuring an adequate supply of undissolved salt at all times. A cell with two metal electrodes polarized by a small DC potential also may be used. (See 4500-Cl.D.7.)

b. Agitator, designed to give adequate agitation at the noble-metal electrode surface to ensure proper sensitivity. Thoroughly clean agitator and exposed electrode system to remove all chlorine- consuming contaminants by immersing them in water containing 1 to 2 mg/L free chlorine for a few minutes. Add KI to the same water and let agitator and electrodes remain immersed for 5 min. After thorough rinsing with chlorine-demand-free water or the sample to be tested, sensitized electrodes and agitator are ready for use. Remove iodide reagent completely from cell.

c. Buret: Commercial titrators usually are equipped with suitable burets (1 mL). Manual burets are available.*

d. Glassware, exposed to water containing at least 10 mg/L chlorine for 3 h or more before use and rinsed with chlorine-demand-free water.

3. Reagents

a. Standard phenylarsine oxide titrant: See 4500-Cl.C.3*a*.

b. Phosphate buffer solution, pH 7: Dissolve 25.4 g anhydrous KH_2PO_4 and 34.1 g anhydrous Na_2HPO_4 in 800 mL distilled water. Add 2 mL sodium hypochlorite solution containing 1% chlorine and mix thoroughly. Protect from sunlight for 2 d. Determine that free chlorine still remains in the solution. Then expose to sunlight until no chlorine remains. If necessary, carry out the final dechlorination with an ultraviolet lamp. Determine that no total chlorine remains by adding KI and measuring with one of the colorimetric tests. Dilute to 1 L with distilled water and filter if any precipitate is present.

c. Potassium iodide solution: Dissolve 50 g KI and dilute to 1 L with freshly boiled and cooled distilled water. Store in the dark in a brown glass-stoppered bottle, preferably in the refrigerator. Discard when solution becomes yellow.

d. Acetate buffer solution, pH 4: See 4500-Cl.C.3*e*.

4. Procedure

a. Sample volume: Select a sample volume requiring no more than 2 mL phenylarsine oxide titrant. Thus, for chlorine concentrations of 2 mg/L or less, take a 200-mL sample; for chlorine levels in excess of 2 mg/L, use 100 mL or proportionately less.

b. Free chlorine: Unless sample pH is known to be between 6.5 and 7.5, add 1 mL pH 7 phosphate buffer solution to produce a pH of 6.5 to 7.5. Titrate with standard phenylarsine oxide titrant, observing current changes on microammeter. Add titrant in progressively smaller increments until all needle movement ceases. Make successive buret readings when needle action becomes sluggish, signaling approach of endpoint. Subtract last very small increment that causes no needle response because of overtitration. Alternatively, use a system involving continuous current measurements and determine endpoint mathematically.

Continue titrating for combined chlorine as described in ¶ *c* below or for the separate monochloramine and dichloramine fractions as detailed in ¶s *e* and *f*.

c. Combined chlorine: To sample remaining from free-chlorine titration add 1.00 mL KI solution and 1 mL acetate buffer solution, in that order. Titrate with phenylarsine oxide titrant to the endpoint, as above. Do not refill buret but simply continue titration after recording figure for free chlorine. Again subtract last increment to give amount of titrant actually used in reaction with chlorine. (If titration was continued without refilling buret, this figure represents total chlorine. Subtracting free chlorine from total gives combined chlorine.) Wash apparatus and sample cell thoroughly to remove iodide ion to avoid inaccuracies when the titrator is used subsequently for a free chlorine determination.

* Kimax 17110-F, 5 mL, Kimble Products, Box 1035, Toledo, OH, or equivalent.

Chlorine, Free

USEPA DPD Method[1] **Method 8021**

0.02 to 2.00 mg/L Cl_2 **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] This product has not been evaluated to test for chlorine and chloramines in medical applications in the United States.

[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

Instrument-specific information

Table 1 shows sample cell and orientation requirements for reagent addition tests, such as powder pillow or bulk reagent tests. Table 2 shows sample cell and adapter requirements for AccuVac Ampul tests. The tables also show all of the instruments that have the program for this test.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for reagent addition

Instrument	Sample cell orientation	Sample cell
DR 6000 DR 3800 DR 2800 DR 2700 DR 1900	The fill line is to the right.	2495402 
DR 5000 DR 3900	The fill line is toward the user.	
DR 900	The orientation mark is toward the user.	2401906 

Table 2 Instrument-specific information for AccuVac Ampuls

Instrument	Adapter	Sample cell
DR 6000 DR 5000 DR 900	—	2427606 
DR 3900	LZV846 (A)	
DR 1900	9609900 or 9609800 (C)	
DR 3800 DR 2800 DR 2700	LZV584 (C)	2122800 

Before starting

Samples must be analyzed immediately after collection and cannot be preserved for later analysis.
Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
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 measurements.
If the test result is over-range, or if the sample temporarily turns yellow after the reagent addition, dilute the sample with a known volume of high quality, chlorine demand-free water and do the test again. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. Additional methods are available to measure chlorine without dilution.
For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option.
An AccuVac Ampule for Blanks can be used to zero the instrument in the AccuVac test procedure.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.
The SwifTest Dispenser for Free Chlorine can be used in place of the powder pillow in the test procedure.

Items to collect

Powder pillows

Description	Quantity
DPD Free Chlorine Reagent Powder Pillows, 10-mL	1
Sample cells. (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	2

Refer to Consumables and replacement items on page 6 for order information.

AccuVac Ampuls

Description	Quantity
DPD Free Chlorine Reagent AccuVac Ampuls	1
Beaker, 50-mL	1
Sample cells (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Stopper for 18-mm tubes and AccuVac Ampuls	1

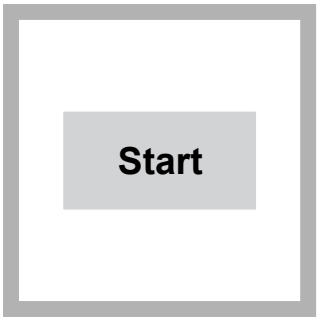
Refer to Consumables and replacement items on page 6 for order information.

Sample collection

- Analyze the samples immediately. The samples cannot be preserved for later analysis.
- Chlorine is a strong oxidizing agent and is unstable in natural waters. Chlorine reacts quickly with various inorganic compounds and more slowly with organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence the decomposition of chlorine in water.
- Collect samples in clean glass bottles. Do not use plastic containers because these can have a large chlorine demand.

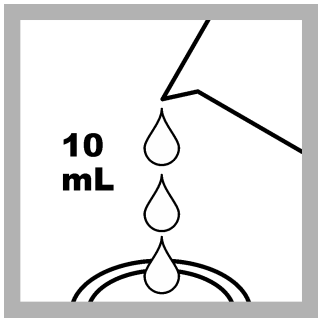
- Pretreat glass sample containers to remove chlorine demand. Soak the containers in a weak bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse fully with deionized or distilled water. If sample containers are rinsed fully with deionized or distilled water after use, only occasional pretreatment is necessary.
- Make sure to get a representative sample. If the sample is taken from a spigot or faucet, let the water flow for at least 5 minutes. Let the container overflow with the sample several times and then put the cap on the sample container so that there is no headspace (air) above the sample.

Powder pillow procedure

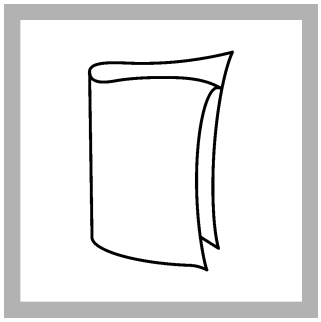


1. Start program **80 Chlorine F&T PP**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.

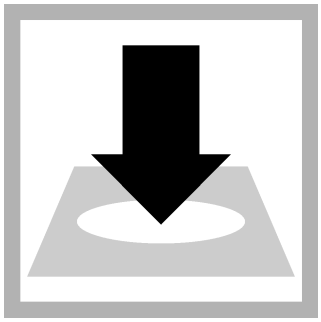
Note: *Although the program name can be different between instruments, the program number does not change.*



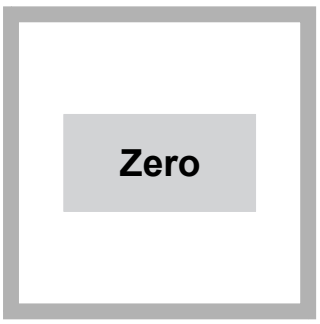
2. Prepare the blank: Fill the sample cell with 10 mL of sample.



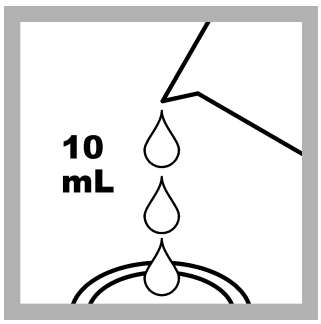
3. Clean the prepared sample cell.



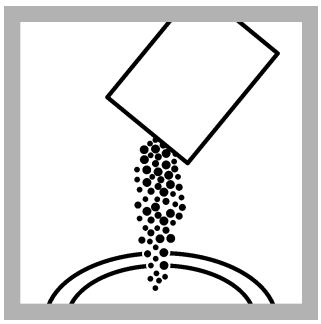
4. Insert the blank into the cell holder.



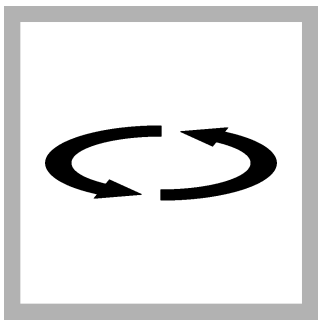
5. Push **ZERO**. The display shows 0.00 mg/L.



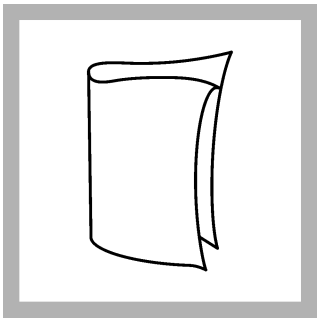
6. Prepare the sample: Fill a second sample cell with 10 mL of sample.



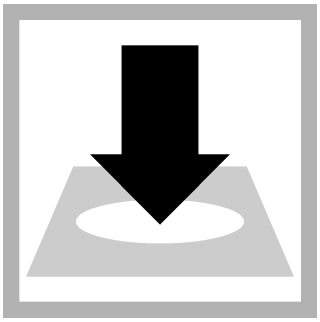
7. Add the contents of one powder pillow to the sample cell.



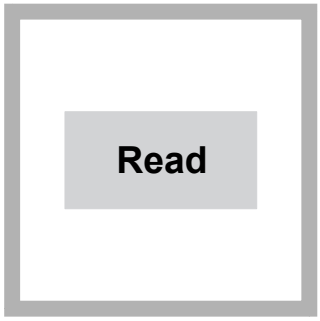
8. Swirl the sample cell for 20 seconds to mix.
A pink color will develop if chlorine is present. Proceed to the next step immediately.



9. Clean the prepared sample cell.

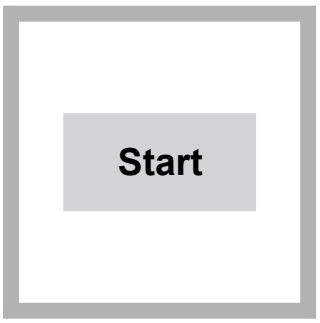


10. Within 60 seconds of the reagent addition, insert the prepared sample into the cell holder.



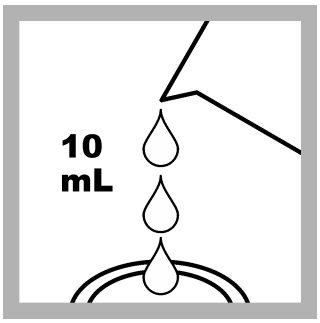
11. Push **READ**. Results show in mg/L Cl_2.

AccuVac Ampul procedure

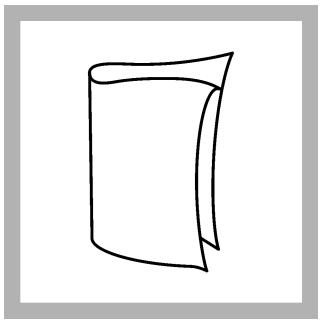


1. Start program **85 Chlorine F&T AV**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.

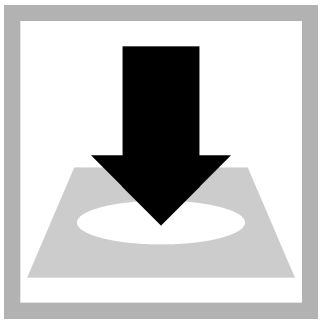
Note: *Although the program name can be different between instruments, the program number does not change.*



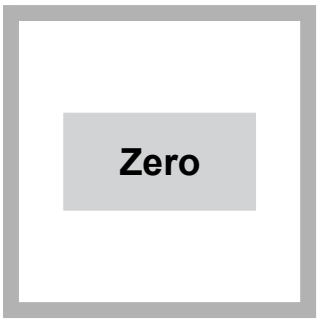
2. Prepare the blank: Fill the sample cell with 10 mL of sample.



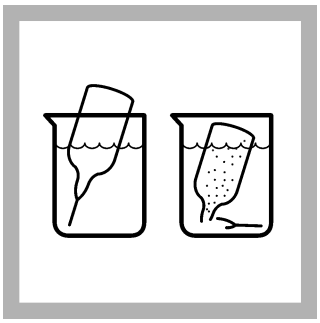
3. Clean the blank sample cell.



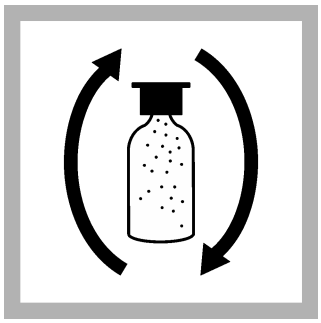
4. Insert the blank into the cell holder.



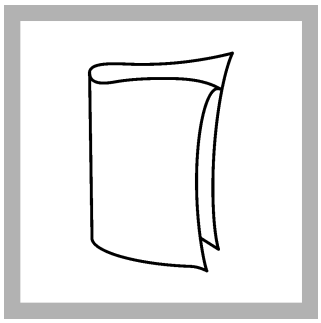
5. Push **ZERO**. The display shows 0.00 mg/L.



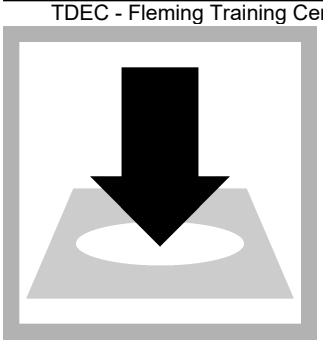
6. Prepare the sample: Collect at least 40 mL of sample in a 50-mL beaker. Fill the AccuVac Ampul with sample. Keep the tip immersed while the AccuVac Ampul fills completely.



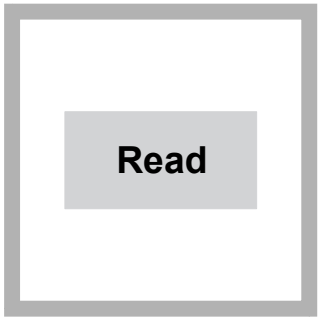
7. Quickly invert the AccuVac Ampul several times to mix.



8. Clean the AccuVac Ampul.



9. Within 60 seconds of the reagent addition, insert the prepared sample AccuVac Ampul into the cell holder.



10. Push **READ**. Results show in mg/L Cl_2.

Interferences

Interfering substance	Interference level
Acidity	More than 150 mg/L $CaCO_3$. The full color may not develop or the color may fade instantly. Adjust to pH 6–7 with 1 N Sodium Hydroxide. Measure the amount to add on a separate sample aliquot, then add the same amount to the sample that is tested. Correct the test result for the dilution from the volume addition.
Alkalinity	More than 250 mg/L $CaCO_3$. The full color may not develop or the color may fade instantly. Adjust to pH 6–7 with 1 N Sulfuric Acid. Measure the amount to add on a separate sample aliquot, then add the same amount to the sample that is tested. Correct the test result for the dilution from the volume addition.
Bromine, Br_2	Positive interference at all levels
Chlorine Dioxide, ClO_2	Positive interference at all levels
Inorganic chloramines	Positive interference 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+})	Pre-treat the sample as follows: **1.** Adjust the sample pH to 6–7. **2.** Add 3 drops of Potassium Iodide (30-g/L) to 10 mL of sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops of Sodium Arsenite (5-g/L) and mix. **5.** Use the test procedure to measure the concentration of the treated sample. **6.** Subtract this result from the result without the treatment 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	Positive interference at all levels
Peroxides	May interfere
Highly buffered samples or extreme sample pH	Can prevent the correct pH adjustment of the sample by the reagents. Sample pre-treatment may be necessary. Adjust to pH 6–7 with acid (Sulfuric Acid, 1.000 N) or base (Sodium Hydroxide, 1.00 N).

Accuracy check

Standard additions method (sample spike)

Use the standard additions method (for applicable instruments) to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L (use mg/L on label)
- Breaker, PourRite Ampules
- Pipet, TenSette®, 0.1–1.0 mL and tips

1. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
2. Go to the Standard Additions option in the instrument menu.
3. Select the values for standard concentration, sample volume and spike volumes.
4. Open the standard solution.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 10-mL portions of fresh sample. Mix well.

   Note: *For AccuVac® Ampuls, add 0.4 mL, 0.8 mL and 1.2 mL of the standard solution to three 50-mL portions of fresh sample.*
6. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.
7. Select **Graph** to compare the expected results to the actual results.

   Note: *If the actual results are significantly different from the expected results, make 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 the results are not within acceptable limits, the sample may contain an interference.*

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% Confidence Interval)	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. The measurement wavelength is 530 nm for spectrophotometers or 520 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
DPD Free Chlorine Reagent Powder Pillow, 10-mL	1	100/pkg	2105569
OR			
DPD Free Chlorine Reagent AccuVac® Ampul	1	25/pkg	2502025

Required apparatus

Description	Quantity/Test	Unit	Item no.
AccuVac Snapper	1	each	2405200
Beaker, 50-mL	1	each	50041H
Stoppers for 18-mm tubes and AccuVac Ampuls	2	6/pkg	173106

Recommended standards

Description	Unit	Item no.
Chlorine Standard Solution, 2-mL PourRite® Ampules, 25–30 mg/L	20/pkg	2630020

Optional reagents and apparatus

Description	Unit	Item no.
AccuVac® Ampul vials for sample blanks	25/pkg	2677925
Ampule Breaker, 2-mL PourRite® Ampules	each	2484600
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
Water, Chlorine-demand Free	500 mL	2641549
Mixing cylinder, graduated, 25-mL	each	2088640
Mixing cylinder, graduated, 50-mL	each	189641
Chlorine Standard Solution, 2-mL PourRite® Ampules, 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
Paper, pH, 0–14 pH range	100/pkg	2601300
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Potassium Iodide, 30-g/L	100 mL	34332
Sodium Arsenite, 5-g/L	100 mL	104732
Sodium Hydroxide Standard Solution, 1.0 N	100 mL MDB	104532
SpecCheck™ Secondary Standard Kit, Chlorine DPD, 0–2.0 mg/L Set	each	2635300
Sulfuric Acid Standard Solution, 1 N	100 mL MDB	127032

Chlorine, Free

USEPA DPD Method[2] — Method 10245[1]

0.05 to 4.00 mg/L Cl_2 (MR) — Powder Pillows

Scope and application: For free chlorine (hypochlorous acid and hypochlorite ion) measurements in water, treated waters, estuary and seawater. This product has not been evaluated to test for chlorine and chloramines in medical applications in the United States.

[1] USEPA accepted for reporting wastewater and drinking water analyses.
[2] Procedure is equivalent to USEPA method 330.5 for wastewater and Standard Method 4500-Cl G for drinking water.

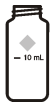
Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows sample cell and adapter requirements for this test.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information

Instrument	Adapter	Sample cell
DR6000	—	2427606 
DR5000	A23618	
DR3900	LZV846 (A)	
DR3800 DR2800 DR2700	LZV584 (C)	
DR1900	9609900 or 9609800 (C)	
DR900	—	2401906 

Before starting

Analyze the samples immediately. The samples cannot be preserved for later analysis.
Install the instrument cap on the DR900 cell holder before ZERO or READ is pushed.
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 measurements.
If the test result is over-range, or if the sample temporarily turns yellow after the reagent addition, dilute the sample with a known volume of high quality, chlorine demand-free water and do the test again. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. Additional methods are available to measure chlorine without dilution.
For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option.
Cold waters can cause condensation on the sample cells during color development. Examine the sample cells for condensation before measurements.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

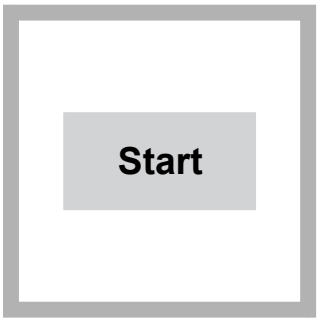
Description	Quantity
DPD Free Chlorine Reagent Powder Pillows, 25-mL	1
Sample cells (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	2

Refer to Consumables and replacement items on page 5 for order information.

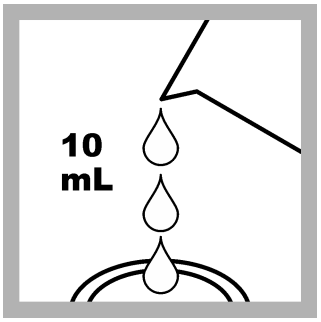
Sample collection

- Analyze the samples immediately. The samples cannot be preserved for later analysis.
- Chlorine is a strong oxidizing agent and is unstable in natural waters. Chlorine reacts quickly with various inorganic compounds and more slowly with organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence the decomposition of chlorine in water.
- Collect samples in clean glass bottles. Do not use plastic containers because these can have a large chlorine demand.
- Pretreat glass sample containers to remove chlorine demand. Soak the containers in a weak bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse fully with deionized or distilled water. If sample containers are rinsed fully with deionized or distilled water after use, only occasional pretreatment is necessary.
- Make sure to get a representative sample. If the sample is taken from a spigot or faucet, let the water flow for at least 5 minutes. Let the container overflow with the sample several times and then put the cap on the sample container so that there is no headspace (air) above the sample.

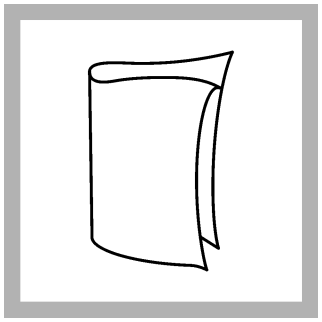
Powder pillow procedure



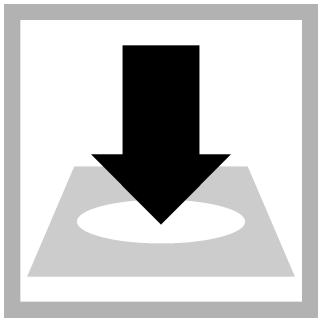
1. Start program **87 Chlorine, F&T PP MR**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



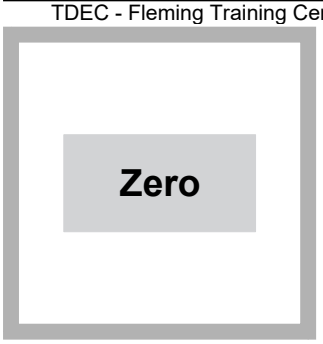
2. Prepare the blank: Fill the sample cell with 10 mL of sample.



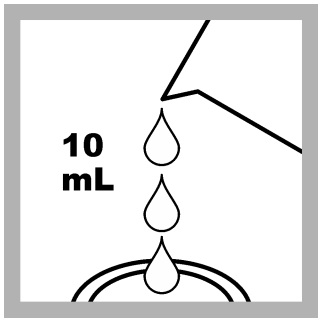
3. Clean the blank sample cell.



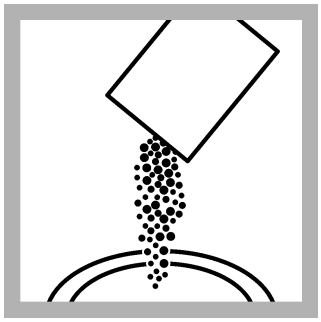
4. Insert the blank into the cell holder.



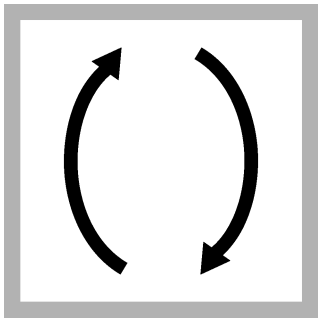
5. Push **ZERO**. The display shows 0.00 mg/L Cl_2.



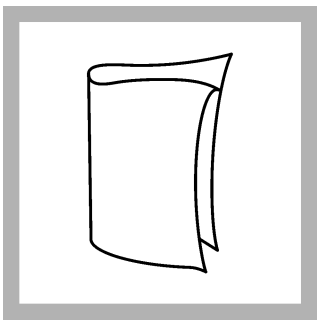
6. Prepare the sample: Fill a second sample cell with 10 mL of sample.



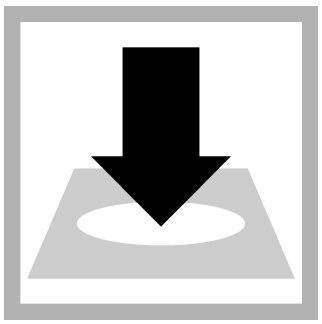
7. Add the contents of one DPD Free Chlorine Powder Pillow for 25-mL sample to the prepared sample cell.



8. Put the stopper on the prepared sample cell. Invert the sample cell several times to mix. A pink color shows if chlorine is present. Go to the next step immediately.



9. Clean the prepared sample cell.



10. Within one minute of the reagent addition, insert the prepared sample into the cell holder.

11. Push **READ**. Results show in mg/L Cl_2.

Interferences

Interfering substance	Interference level
Acidity	More than 150 mg/L $CaCO_3$. The full color may not develop or the color may fade instantly. Adjust to pH 6–7 with 1 N Sodium Hydroxide. Measure the amount to add on a separate sample aliquot, then add the same amount to the sample that is tested. Correct the test result for the dilution from the volume addition.
Alkalinity	More than 250 mg/L $CaCO_3$. The full color may not develop or the color may fade instantly. Adjust to pH 6–7 with 1 N Sulfuric Acid. Measure the amount to add on a separate sample aliquot, then add the same amount to the sample that is tested. Correct the test result for the dilution from the volume addition.
Bromine, Br_2	Positive interference at all levels
Chlorine Dioxide, ClO_2	Positive interference at all levels
Inorganic chloramines	Positive interference at all levels
Chloramines, organic	May interfere in the result for total chlorine analysis
Hardness	No effect at less than 1000 mg/L as $CaCO_3$

Interfering substance	Interference level
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	Pre-treat the sample as follows: **1.** Adjust the sample pH to 6–7. **2.** Add 3 drops of Potassium Iodide (30-g/L) to 10 mL of sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops of Sodium Arsenite (5-g/L) and mix. **5.** Use the test procedure to measure the concentration of the treated sample. **6.** Subtract this result from the result without the treatment to obtain the correct chlorine concentration.
Ozone	Positive interference at all levels
Peroxides	May interfere
Highly buffered samples or extreme sample pH	Can prevent the correct pH adjustment (of the sample) by the reagents. Sample pretreatment may be necessary. Adjust to pH 6–7 with acid (Sulfuric Acid, 1.000 N) or base (Sodium Hydroxide, 1.00 N).

Monochloramine interference

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 shown in Table 2 (1 minute test time). Measure the monochloramine levels with method 10200 for Chloramine (Mono) and Free Ammonia.

Table 2 Monochloramine interference at different sample temperatures

NH_2Cl (as Cl_2)	5 °C (41 °F)	10 °C (50 °F)	20 °C (68 °F)	30 °C (83 °F)
1.2 mg/L	0.15	0.19	0.30	0.29
2.2 mg/L	0.35	0.38	0.55	0.61
3.2 mg/L	0.38	0.56	0.69	0.73

Accuracy check

Standard additions method (sample spike)

Use the standard additions method (for applicable instruments) to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 (or 50–75) mg/L
- Ampule breaker
- Pipet, TenSette®, 0.1–1.0 mL and tips

1. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
2. Go to the Standard Additions option in the instrument menu.
3. Select the values for standard concentration, sample volume and spike volumes.
4. Open the standard solution.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 10-mL portions of fresh sample. Mix well.
6. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.

7. Select **Graph** to compare the expected results to the actual results.

   Note: *If the actual results are significantly different from the expected results, make 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 the results are not within acceptable limits, the sample may contain an interference.*

Verification of on-line analyzers

This procedure can be used to meet the requirements of USEPA Method 334.0 - Determination of Residual Chlorine in Drinking Water Using an On-line Chlorine Analyzer. The procedure and requirements for compliance with EPA Method 334.0 can be downloaded directly from http://www.hach.com/method334.

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
87	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, the intensity of which is proportional to the chlorine concentration. The measurement wavelength is 530 nm for spectrophotometers or 520 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
DPD Free Chlorine Reagent Powder Pillow, 25 mL	1	100/pkg	1407099
Sample cell, 10-mL round, 25 mm x 60 mm	1	6/pkg	2427606

Recommended standards

Description	Unit	Item no.
Chlorine Standard Solution, 2-mL PourRite® Ampules, 25–30 mg/L	20/pkg	2630020
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
PourRite® Ampule Breaker, 2-mL	each	2484600
Chlorine Standard Solution, 2-mL PourRite® Ampules, 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	Item no.
Sodium Hydroxide Standard Solution, 1.0 N	100 mL MDB	104532
Sulfuric Acid Standard Solution, 1 N	100 mL MDB	127032
Potassium Iodide, 30-g/L	100 mL	34332
Sodium Arsenite, 5-g/L	100 mL	104732
Pipet, TenSette®, 0.1–1.0 mL	each	1970001

Optional reagents and apparatus (continued)

Description	Unit	Item no.
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Paper, pH, 0–14 pH range	100/pkg	2601300
DPD Free Chlorine Reagent Powder Pillows, 25 mL	1000/pkg	1407028
SpecCheck™ Secondary Standard Kit, Chlorine DPD, MR	each	2980500
Water, organic-free	500 mL	2641549
Freechlor F Reagent Solution	50 mL SCDB	2964926
Monochlor F Reagent Powder Pillows	100/pkg	2802299

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05/2019, Edition 11

Chlorine, Total

DOC316.53.01027

USEPA DPD Method[1]

Method 8167

0.02 to 2.00 mg/L Cl_2

Powder Pillows or AccuVac® Ampuls

Scope and application: For testing residual chlorine and chloramines in water, wastewater, estuary water and seawater; USEPA-accepted for reporting for drinking and wastewater analyses.[2] This product has not been evaluated to test for chlorine and chloramines in medical applications in the United States.

[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 and wastewater analysis.

Test preparation

Instrument-specific information

Table 1 shows sample cell and orientation requirements for reagent addition tests, such as powder pillow or bulk reagent tests. Table 2 shows sample cell and adapter requirements for AccuVac Ampul tests. The tables also show all of the instruments that have the program for this test.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for reagent addition

Instrument	Sample cell orientation	Sample cell
DR 6000 DR 3800 DR 2800 DR 2700 DR 1900	The fill line is to the right.	2495402 
DR 5000 DR 3900	The fill line is toward the user.	
DR 900	The orientation mark is toward the user.	2401906 

Table 2 Instrument-specific information for AccuVac Ampuls

Instrument	Adapter	Sample cell
DR 6000 DR 5000 DR 900	—	2427606 
DR 3900	LZV846 (A)	
DR 1900	9609900 or 9609800 (C)	
DR 3800 DR 2800 DR 2700	LZV584 (C)	2122800 

Before starting

Analyze the samples immediately. The samples cannot be preserved for later analysis.
Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
If the test result is over-range, or if the sample temporarily turns yellow after the reagent addition, dilute the sample with a known volume of high quality, chlorine demand-free water and do the test again. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. Additional methods are available to measure chlorine without dilution.
For chloramination disinfection control, use one of the available Chloramine (Mono) methods.
For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.
The SwifTest Dispenser for Total Chlorine can be used in place of the powder pillow in the test procedure. One dispensation is equal to one powder pillow for 10-mL samples.
An AccuVac Ampul for Blanks can be used to zero the instrument in the AccuVac test procedure.

Items to collect

Powder pillows

Description	Quantity
DPD Total Chlorine Reagent Powder Pillow, 10-mL	1
Sample cells. (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	2

Refer to Consumables and replacement items on page 6 for order information.

AccuVac Ampuls

Description	Quantity
DPD Total Chlorine Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample cells (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Stopper for 18-mm tubes and AccuVac Ampuls	1

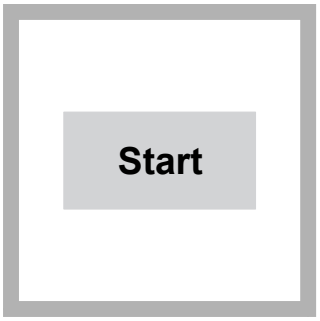
Refer to Consumables and replacement items on page 6 for order information.

Sample collection

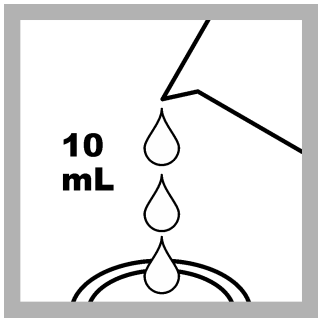
- Analyze the samples immediately. The samples cannot be preserved for later analysis.
- Chlorine is a strong oxidizing agent and is unstable in natural waters. Chlorine reacts quickly with various inorganic compounds and more slowly with organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence the decomposition of chlorine in water.
- Collect samples in clean glass bottles. Do not use plastic containers because these can have a large chlorine demand.

- Pretreat glass sample containers to remove chlorine demand. Soak the containers in a weak bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse fully with deionized or distilled water. If sample containers are rinsed fully with deionized or distilled water after use, only occasional pretreatment is necessary.
- Make sure to get a representative sample. If the sample is taken from a spigot or faucet, let the water flow for at least 5 minutes. Let the container overflow with the sample several times and then put the cap on the sample container so that there is no headspace (air) above the sample.

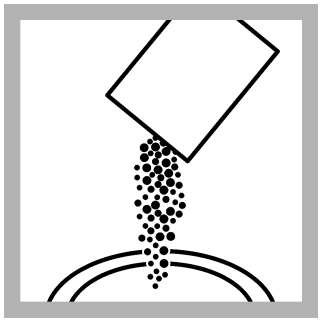
Powder pillow procedure



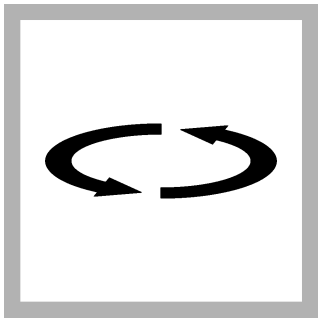
1. Start program **80 Chlorine F&T PP**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



2. Fill a sample cell with 10 mL of sample.



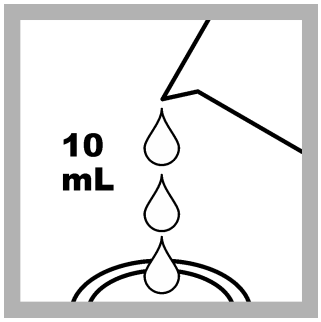
3. Prepare the sample: Add the contents of one powder pillow to the sample cell.



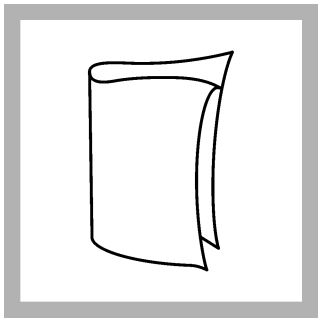
4. Swirl the sample cell for 20 seconds to mix.
A pink color shows if chlorine is present in the sample.

5. Start the instrument timer. A 3-minute reaction time starts.

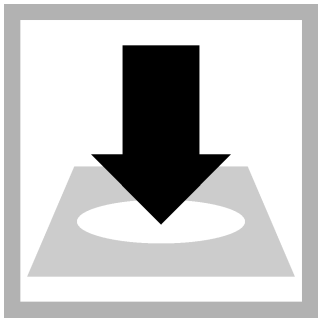
Prepare the sample blank and set the instrument to zero during the reaction time.



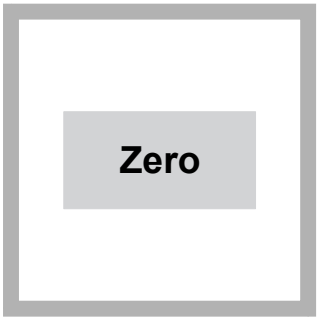
6. Prepare the blank: Fill a second sample cell with 10 mL of sample.



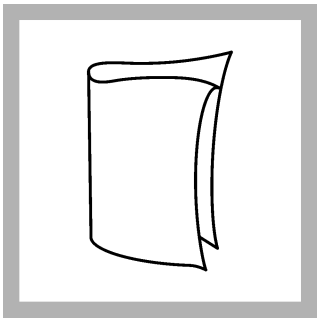
7. Clean the blank sample cell.



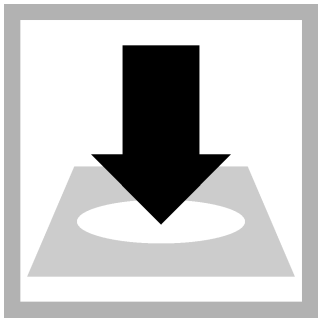
8. Insert the blank into the cell holder.



9. Push **ZERO**. The display shows 0.00 mg/L Cl_2.



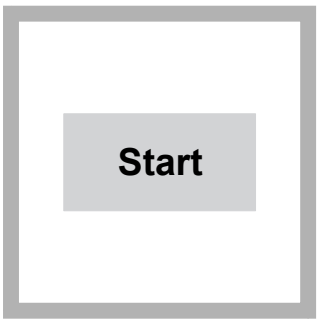
10. Clean the prepared sample cell.



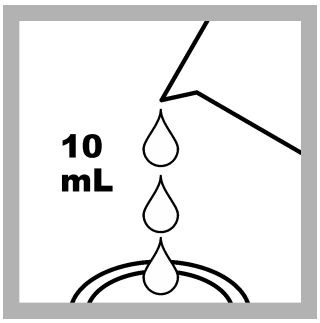
11. Within 3 minutes after the timer expires, insert the prepared sample into the cell holder.

12. Push **READ**. Results show in mg/L Cl_2.

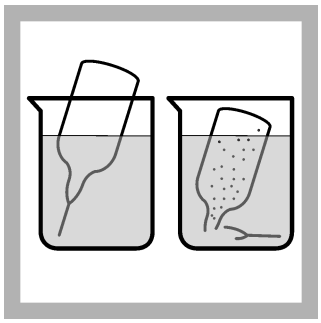
AccuVac Ampul procedure



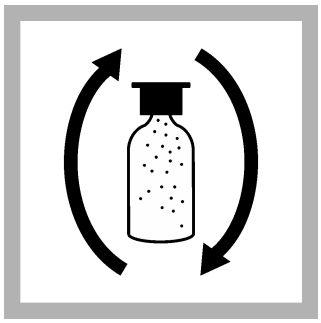
1. Start program **85 Chlorine F&T AV**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



2. Prepare the blank: Fill the sample cell with 10 mL of sample.



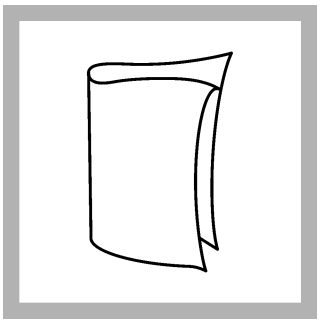
3. Prepare the sample: Collect at least 40 mL of sample in a 50-mL beaker. Fill the AccuVac Ampul with sample. Keep the tip immersed while the AccuVac Ampul fills completely.



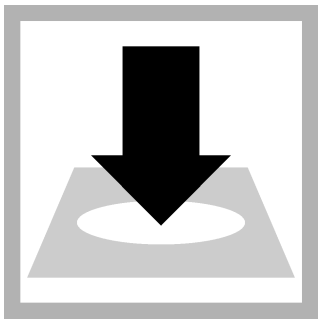
4. Quickly invert the AccuVac Ampul several times to mix.

5. Start the instrument timer. A 3-minute reaction time starts.

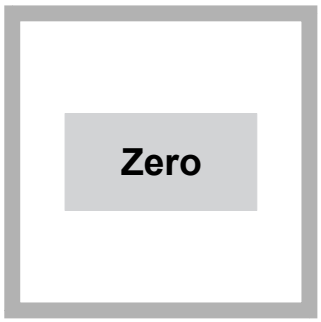
Prepare the sample blank and set the instrument to zero during the reaction time.



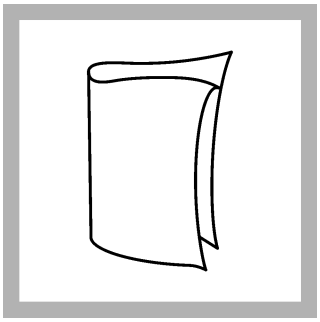
6. Clean the blank sample cell.



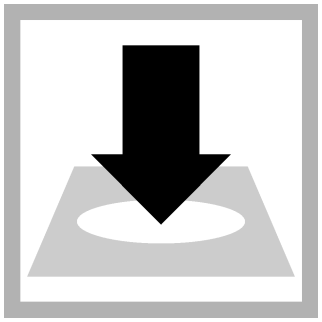
7. Insert the blank into the cell holder.



8. Push **ZERO**. The display shows 0.00 mg/L Cl_2.



9. Clean the AccuVac Ampul.



10. Within 3 minutes after the timer expires, insert the prepared sample AccuVac Ampul into the cell holder.

11. Push **READ**. Results show in mg/L Cl_2.

Interferences

Interfering substance	Interference level
Acidity	More than 150 mg/L $CaCO_3$. The full color may not develop or the color may fade instantly. Adjust to pH 6–7 with 1 N Sodium Hydroxide. Measure the amount to add on a separate sample aliquot, then add the same amount to the sample that is tested. Correct the test result for the dilution from the volume addition.
Alkalinity	More than 250 mg/L $CaCO_3$. The full color may not develop or the color may fade instantly. Adjust to pH 6–7 with 1 N Sulfuric Acid. Measure the amount to add on a separate sample aliquot, then add the same amount to the sample that is tested. Correct the test result for the dilution from the volume addition.
Bromine, Br_2	Positive interference at all levels
Chlorine Dioxide, ClO_2	Positive interference at all levels
Inorganic chloramines	Positive interference at all levels
Chloramines, organic	May interfere in the result for total chlorine analysis
Hardness	No effect at less than 1000 mg/L as $CaCO_3$
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	Pre-treat the sample as follows: **1.** Adjust the sample pH to 6–7. **2.** Add 3 drops of Potassium Iodide (30-g/L) to 10 mL of sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops of Sodium Arsenite (5-g/L) and mix. **5.** Use the test procedure to measure the concentration of the treated sample. **6.** Subtract this result from the result without the treatment to obtain the correct chlorine concentration.
Ozone	Positive interference at all levels
Peroxides	May interfere
Highly buffered samples or extreme sample pH	Can prevent the correct pH adjustment (of the sample) by the reagents. Sample pretreatment may be necessary. Adjust to pH 6–7 with acid (Sulfuric Acid, 1.000 N) or base (Sodium Hydroxide, 1.00 N).

Accuracy check

Standard additions method (sample spike)

Use the standard additions method (for applicable instruments) to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- Chlorine Standard Solution, 2-mL PourRite® Ampule, 25–30 mg/L (use mg/L on label)
- Breaker, PourRite Ampules
- Pipet, TenSette®, 0.1–1.0 mL and tips

1. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
2. Go to the Standard Additions option in the instrument menu.
3. Select the values for standard concentration, sample volume and spike volumes.
4. Open the standard solution.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 10-mL portions of fresh sample. Mix well.

   Note: *For AccuVac® Ampuls, add 0.4 mL, 0.8 mL and 1.2 mL of the standard solution to three 50-mL portions of fresh sample.*
6. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.
7. Select **Graph** to compare the expected results to the actual results.

   Note: *If the actual results are significantly different from the expected results, make 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 the results are not within acceptable limits, the sample may contain an interference.*

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% Confidence Interval)	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 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 react with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total chlorine concentration.

To find 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. The measurement wavelength is 530 nm for spectrophotometers or 520 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
DPD Total Chlorine Reagent Powder Pillow, 10 mL	1	100/pkg	2105669
OR			
DPD Total Chlorine Reagent AccuVac® Ampul	1	25/pkg	2503025

Required apparatus

Description	Quantity/Test	Unit	Item no.
AccuVac Snapper	1	each	2405200
Beaker, 50 mL	1	each	50041H
Stoppers for 18-mm tubes and AccuVac Ampuls	2	6/pkg	173106

Recommended standards

Description	Unit	Item no.
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
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

Optional reagents and apparatus

Description	Unit	Item no.
AccuVac® Ampul vials for sample blanks	25/pkg	2677925
Ampule Breaker, 2-mL PourRite® Ampules	each	2484600
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
Water, Chlorine-demand Free	500 mL	2641549
Mixing cylinder, graduated, 25-mL	each	2088640
Mixing cylinder, graduated, 50 mL	each	189641
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
Paper, pH, 0–14 pH range	100/pkg	2601300
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Potassium Iodide, 30-g/L	100 mL	34332
Sodium Arsenite, 5-g/L	100 mL	104732
Sodium Hydroxide Standard Solution, 1.0 N	100 mL MDB	104532
SpecCheck™ Secondary Standard Kit, Chlorine DPD, 0–2.0 mg/L Set	each	2635300
Sulfuric Acid Standard Solution, 1 N	100 mL MDB	127032
Water, deionized	4 L	27256

Chlorine, Free and Total TNTplus

DOC316.53.01248

DPD Method[1] **Method 10231 (Free Chlorine) and Method 10232 (Total Chlorine)**

0.05 to 2.00 mg/L Cl_2 **TNTplus™ 866/867**

Scope and application: For drinking water, wastewater and pool water. This product has not been evaluated to test for chlorine and chloramines in medical applications in the United States.

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

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the applicable instruments that can use TNTplus vials.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for TNTplus vials

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800	—	LZV646
DR 1900	9609900 or 9609800 (A)	—

Before starting

DR 3900, DR 3800, DR 2800: Install the light shield in Cell Compartment #2 before this test is started.
Review the safety information and the expiration date on the package.
Analyze the samples immediately. The samples cannot be preserved for later analysis.
The recommended sample pH is 3–10.
The recommended temperature for samples and reagents is 15–25 °C (59–77 °F).
The recommended temperature for reagent storage is 2–8 °C (35–46 °F).
To sequentially measure free and total chlorine, complete the free chlorine procedure, then start the total chlorine procedure at step 2.
DR 1900: Go to All Programs>LCK or TNTplus Methods>Options to select the TNTplus number for the test. Other instruments automatically select the method from the barcode on the vial.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

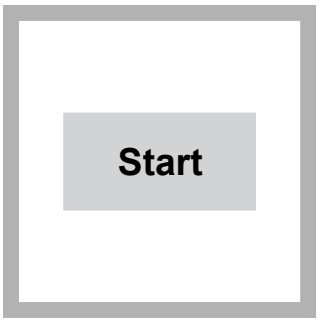
Description	Quantity
Chlorine Free (TNT866) or Total (TNT867) Reagent Set	1
Beaker, 50 mL	1

Refer to Consumables and replacement items on page 4 for order information.

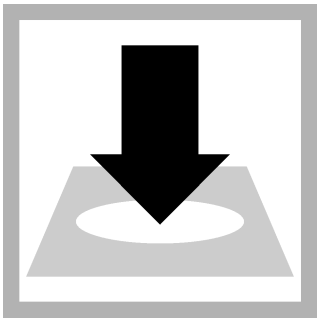
Sample collection

- Analyze the samples immediately. The samples cannot be preserved for later analysis.
- Chlorine is a strong oxidizing agent and is unstable in natural waters. Chlorine reacts quickly with various inorganic compounds and more slowly with organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence the decomposition of chlorine in water.
- Collect samples in clean glass bottles. Do not use plastic containers because these can have a large chlorine demand.
- Pretreat glass sample containers to remove chlorine demand. Soak the containers in a weak bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse fully with deionized or distilled water. If sample containers are rinsed fully with deionized or distilled water after use, only occasional pretreatment is necessary.
- Make sure to get a representative sample. If the sample is taken from a spigot or faucet, let the water flow for at least 5 minutes. Let the container overflow with the sample several times and then put the cap on the sample container so that there is no headspace (air) above the sample.

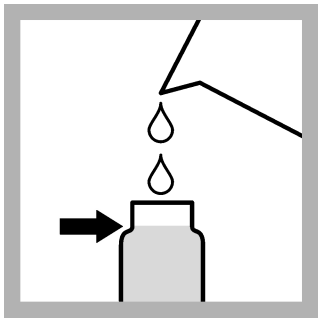
Test procedure—free chlorine



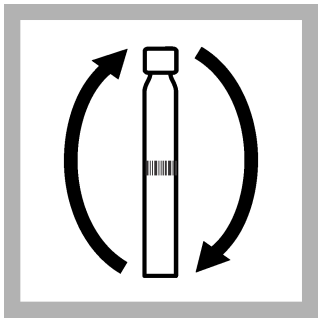
1. DR 1900 only: Select program 866. Refer to Before starting on page 1.



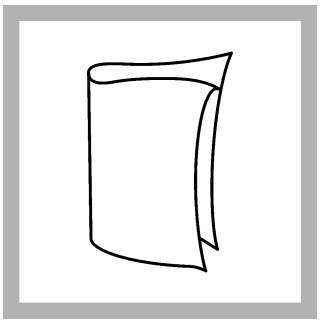
2. Insert the Zero vial into the cell holder. DR 1900 only: Push **ZERO**. The instrument zero is set.



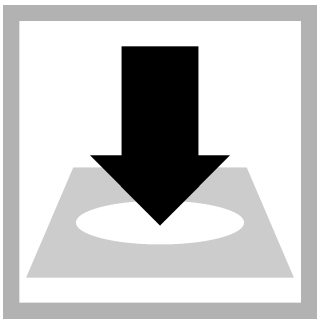
3. Complete this procedure within 1 minute: Fill the sample vial with sample to the throat of the vial.



4. Tighten the cap on the vial and invert the vial 2–3 times. Carefully rotate the vial to remove air bubbles. Do not shake.

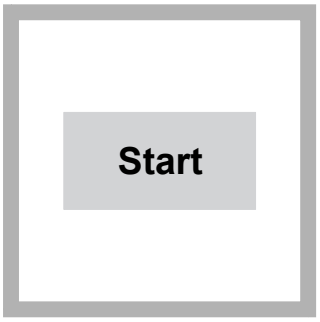


5. Clean the vial.

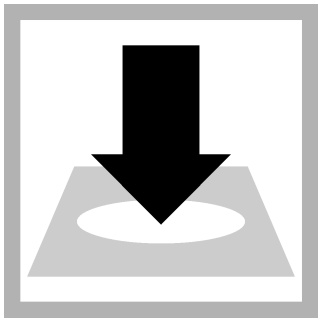


6. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in mg/L Cl_2.

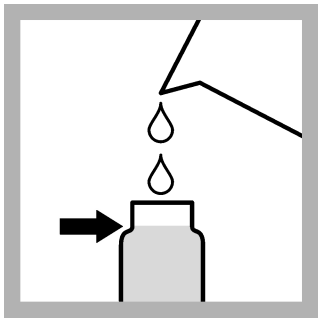
Test procedure—total chlorine



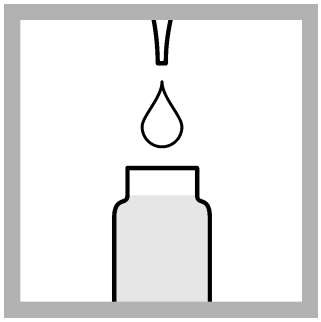
1. DR 1900 only: Select program 867. Refer to Before starting on page 1.



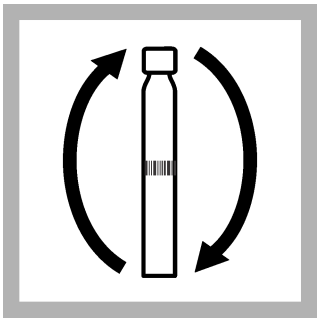
2. Insert the Zero vial into the cell holder. DR 1900 only: Push **ZERO**. The instrument zero is set.



3. Fill the sample vial with sample to the throat of the vial.

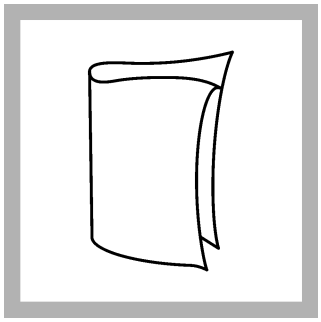


4. Immediately add 1 drop of potassium iodide Solution A.

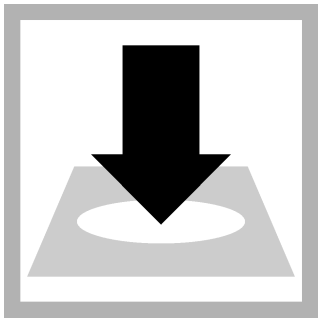


5. Tighten the cap on the vial and invert the vial 2–3 times. Carefully rotate the vial to remove air bubbles. Do not shake.

6. Start the reaction time of 3 minutes.



7. When the timer expires, clean the vial.



8. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in mg/L Cl_2.

Interferences

Interfering substance	Interference level
Color	Can cause high results. To make a correction for the interference, measure a sample blank.
Oxidizing agents	All oxidizing agents (e.g., ozone, iodine, chlorine dioxide, manganese dioxide and chromate) react with the indicator and are included in the results. If a large number of oxidizing agents shows, some or all of the indicator changes to a colorless form. The test result will be negative or within the range of the test.
pH	The sample pH should be between pH 3 and pH 10.
Turbidity	Can cause high results. To make a correction for the interference, measure a sample blank.

Sample blanks

If the sample has color or turbidity, measure a sample blank to correct the test result for the interference.

Items to collect:

- TNTplus 919 sample blank vial

1. Do the test procedure.
2. Put the sample in the sample blank vial. Fill to the neck of the sample blank vial.
3. Wipe the sample blank vial clean, then put it into the cell holder. If applicable, the instrument reads the barcode of the sample blank vial and subtracts the value from the initial test result.

Accuracy check

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 50–75 mg/L Chlorine Standard Solution (use mg/L on label)
- 100-mL volumetric flask, Class A
- Ampule breaker
- Pipet, adjustable volume, 1.0–5.0 mL and pipet tip
- Deionized water

1. Prepare a 1.3-mg/L (approximate) chlorine standard solution as follows:
   a. Use a pipet to add 2.0 mL of the chlorine standard solution into the volumetric flask. First add approximately 50 mL of deionized water to the flask. Multiply the concentration that is shown on the chlorine standard solution label by 0.02 to find the concentration of the prepared standard solution.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the prepared standard solution.
3. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

Summary of Method

Chlorine can show in water as free chlorine and as combined chlorine. Both forms can exist in the same water and can be determined together as total chlorine. Free chlorine shows 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 react with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color that is proportional to the chlorine concentration. The measurement wavelength is 515 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
Free Chlorine TNT 866 Reagent Set	1	24/pkg	TNT866
Free and Total Chlorine TNT 867 Reagent Set	1	24/pkg	TNT867

Required apparatus

Description	Quantity/test	Unit	Item no.
Beaker, 50 mL	1	each	50041H
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Light shield, DR 3900	1	each	LZV849

Recommended standards

Description	Unit	Item no.
Chlorine Standard Solution, 2-mL PourRite® Ampules, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L	16/pkg	1426810
Chlorine Standard Solution, 2-mL PourRite® Ampule, 25-30 mg/L	20/pkg	2630020

Optional reagents and apparatus

Description	Unit	Item no.
Ampule Breaker, 2-mL PourRite® Ampules	each	2484600
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
Flask, volumetric, Class A, 100 mL, glass	each	1457442
Pipet, adjustable volume, 1.0–5.0 mL	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	75/pkg	BBP068
Sample blank vials for TNTplus™ methods	5/pkg	TNT919
Sampling bottle with cap, low density polyethylene, 500-mL	12/pkg	2087079
Water, deionized	4 L	27256

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:

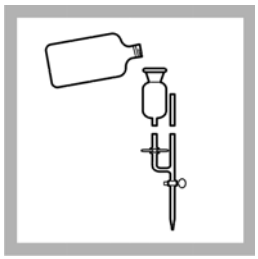
Chlorine can be lost from the sample during sample collection. Review the precautions in *Sample collection, preservation and storage* before the test is started.
Use only a 50-mm stir bar. The wrong size can cause the loss of chlorine, unstable readings and loss of method sensitivity, especially when 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.

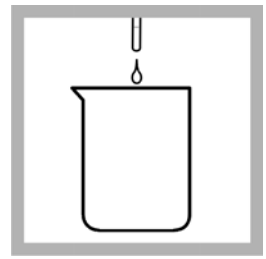
Buret titration



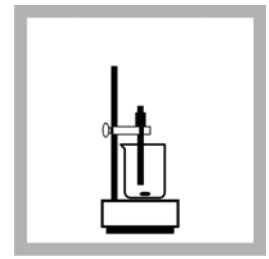
1. Fill the 5-mL automatic buret to the zero mark with 0.00564 N Phenylarsine Oxide (PAO) Solution.

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.

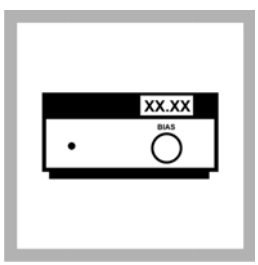


3. If the pH is less than 6 or greater than 7.5, add 1.0 mL of pH 7 Phosphate Buffer Solution to make the prepared sample.



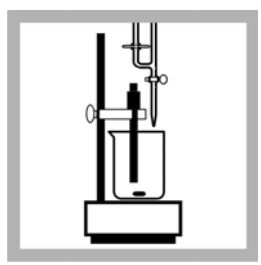
4. Place the beaker of prepared sample on the TitraStir titration stand and turn on the stirring motor. Put the tip of the probe fully into the prepared sample. The platinum wires must be submerged.

If a stir plate other than the TitraStir® is used, set the speed for moderate mixing. Do not adjust the speed after this point.

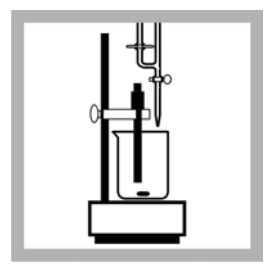


5. Turn the BIAS control knob to adjust the value on the display to approximately 1.00.

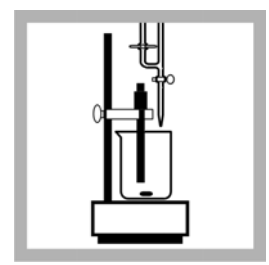
The BIAS adjustment controls the slope of the titration curve. The actual value is not important. Only the relative value as the titration continues is important. A precise adjustment is not necessary.



6. Dispense the titrant into the beaker in small increments while monitoring the values on the Amperometric Titrator. The values will decrease.

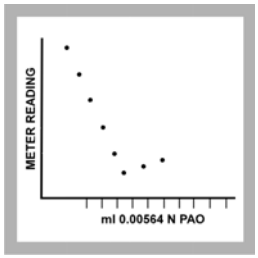


7. Continue dispensing slowly. Near the end point of the titration, write down the value on the display and the corresponding total volume of titrant that was added. Read the volume to the nearest 0.01 mL. Add a small amount of titrant and wait several seconds for a stable value. Write down the value.

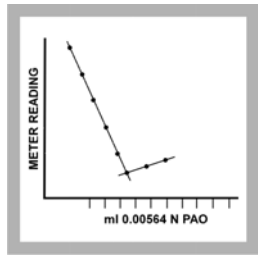


8. Continue the titration by recording at least three points on the downward sloping curve and at least three points after the end point has been reached. The value on the display will not change significantly after the end point.

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

Total Residual Chlorine, SM 4500-Cl G, 22nd edition (2000) – DPD Colorimetric Method

Minimum Detectable Concentration – 4500-Cl G.1.c. – approximately 10 μg/L (0.010 mg/L)

Initial Demonstration of Capability (DOC)

- 4020 B.1.a. - each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – each operator running this test needs to analyze 4 samples of a chlorine standard or potassium permanganate ($KMnO_4$) at a concentration of 0.5 mg/L**
  - **Keep a folder for each analyst, keep a copy here**
  - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
  - **Recommend backup analyst do this once a year.**
  - **Only good for that type of instrument you are using at that plant. If you have a backup instrument made by a different manufacturer or you work at another plant with a different make/model, you would need another DOC.**
    - **DOCs demonstrate you are proficient with that one instrument.**

Method Detection Limit (MDL)

- 1020 B. 4 – As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level (5 x 0.010 mg/L = 0.050 mg/L).
  - Ideally, prepare and analyze at least seven (7) portions of this solution over a 3-day period to ensure that the MDL determination is more representative of routine measurements as performed in the laboratory.
  - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD (relative standard deviation) values ≤ 20%.
- 4020 B.1.b. – Verify MDL at least ***annually***.
  - Ideally use pooled data from several analysts rather than data from one analyst.
- **Real people language – have several operators, who run this test, analyze chlorine standards or Potassium Permanganate ($KMnO_4$) at a concentration of 0.05 mg/L over several days with a total of at least 7 samples**
  - **Joe analyzes 3 samples on Monday**
  - **Bob analyzes 3 samples on Tuesday**
  - **Mary analyzes 3 samples on Wednesday**
- **Run this once a year**

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves.
- 4020.B.2.a. – Calibrate initially with at least one blank and three calibration standards.
  - The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
  - The back-calculated and true concentrations should agree within ± 10%.

- **Real people language – prepare a set of chlorine standards or potassium permanganate ($KMnO_4$) in accordance with Guidance for Secondary Stds use in Calibration 10-19-2012 monthly.**

Method Blank

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure.
- 4020 B.2.d. – Include at least one method blank ***daily*** or with each batch of 20 or fewer samples, whichever is more frequent.
    - If any method blanks measurements are at or above the reporting level, take immediate corrective action.
- **Real people language – analyze distilled water as a sample by adding a DPD powder pillow and waiting the 3-6 minutes before reading**
    - **Target value is less than detection level**
    - **Do this every day you analyze total residual chlorine**

Laboratory Fortified Blank (LFB)

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.
    - Sample batch = 5% basis = 1 every 20 samples
    - Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve.
- 4020 B.2.e. – Calculate percent recovery, plot control charts and determine control limits
- **Real people language – analyze chlorine standard or potassium permanganate sample at a concentration of 0.5 mg/L**
    - **Run on a 5% basis, see batch size for more information**

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 4020 B.2.f. – Randomly select routine samples to be analyzed twice.
    - Process duplicate sample independently through the entire sample preparation and analysis.
    - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
- **Real people language – on a 5% basis (1 for every 20 samples) analyze 2 samples for chlorine, after reading one, pour out sample and start with a fresh sample**
    - **First sample is result, second sample is duplicate**
    - **Target value is to get close to the first value and have a small RPD**

Continuing Calibration Verification (CCV)

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
    - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.

- For the calibration verification to be valid, check standards must not exceed 10% of its true value, and calibration blank results must not be greater than one-half the reporting level

- **Real people language**
  - **Read Secondary Standards in accordance with Guidance for Secondary Stds use in Calibration 10-19-2012 daily.**
  - **OR run a chlorine or potassium permanganate standard daily.**

Control Charts – 1020 B.13.

Corrective Action - 1020 B.5., B.8,. & B.15.

QC Acceptance Criteria

- Blanks < reporting limits
- LFB ± 15%
- ICV/CCV ± 10%
- RPD± 20%

Batch Size

- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
  - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would suggest twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
  - If sampling only once a month, need to run QC once a month.

Calculations

- % Recovery for LFB
  - = $\frac{\text{LFB concentration}}{\text{Expected concentration}}$ X 100%
- RPD – relative percent differences for duplicates and LFM/LFMD
  - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}}$ X 100%

Chlorine Lab
Bench Sheet

Sample Name	Reading (mg/L)	Spike (r^2= ??)
Sample 1 (low range)		
Sample 2 (medium range)		
Sample 3 (total Cl_2)		
TNT – Free Cl_2		
TNT - Total Cl_2		

The Use of Secondary Standards
for Spectrophotometer/Colorimeter Calibration

Secondary standards (gel standards) are specifically designed to verify the instrument's calibration and to check the instrument's performance. They are not intended to be used to create calibration curves or to calibrate the instrument. Because the DPD reagent cannot be mixed with the gel standards, the quality and the reaction time of the reagent cannot be assessed. For this reason gel standards cannot take the place of primary standards.

The analyst is responsible for the following:

- Preparing the calibration curve for each instrument **once per month** at a minimum, with chlorine standards or potassium permanganate (see instructions below for KMnO4) before the use of new DPD reagents, or the use of new gel standards
- Recording reagent lot #'s for reagents and standards
- Recording calibration concentrations
- Verified the calibration curve using a minimum of one blank and two gel standards that bracket the expected sample concentration
- Recording all verification data

STOCK STANDARD SOLUTION

0.891 grams of reagent grade $KMnO_4$ in 1000 mL vol. flask made to mark with deionized water. Deionized water must never be stored in plastic containers or exposed to airborne contamination. Store the stock solution in amber bottle in a cool area. The typical shelf life of the stock solution is six (6) months. If solids appear in the solution, **do not use.**

*****Avoid leaving the cap off for extended periods of time and avoid contamination.*****

INTERMEDIATE (WORKING) STANDARD SOLUTION (10 mg/L)

10 mL of *STOCK* made in *1000* mL vol. flask made to mark with deionized water. The flask should be labeled with the name, $KMnO_4$, date of preparation, initials of who made it.
This information should also be entered into a logbook.
The intermediate stock solution should be stable for approximately 5 days if kept cool and away from light.

Care should be taken that the pipette and glassware are clean and thoroughly rinsed with deionized water to avoid contamination. Store only in glass container (preferably amber glass) never in plastic containers. The working solution should be remade if solids appear in the bottom of the container.

CALIBRATION STANDARD SOLUTIONS

If using KMnO4, four to five calibration standard solutions should be made, according to the table below, to create a calibration curve ***once per month*** at a minimum. The correlation coefficient (r) of the curve should be 0.995 or higher. This curve is then used to check or calibrate the instrument. Gel standards are run against the curve and must meet the manufacturer's published acceptance criteria for the specific instrument being used.
The working solution should be stable for approximately 2 hours if chlorine demand-free water is used.

A target value (e.g. permit value for a facility) should be known and three gel standards, 0.00 mg/L, blank, and two other standards (a low and a high standard) that bracket the target value should be chosen.

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
20 mL (vol. Pipette) to **100** mL (vol. flask)	**2.0** mg/L
10 mL (vol. Pipette) to **100** mL (vol. flask)	**1.0** mg/L
5 mL (vol. Pipette) to **100** mL (vol. flask)	**0.5** mg/L
1 mL (vol. Pipette) to **100** mL (vol. flask)	**0.1** mg/L
1 mL (vol. Pipette) to **200** mL (vol. flask)	**0.05** mg/L
1 mL (vol. Pipette) to **500** mL (vol. flask)	**0.02** mg/L
100 mL of deionized water	**0.00** mg/L

Chlorine QA/QC Bench sheet

mL Working Standard Diluted with Deionized Water	Chlorine Equivalent	Actual Reading
20 mL (vol. pipet) to 100 mL (vol. flask)	2.0 mg/L	
10 mL (vol. pipet) to 100 mL (vol. flask)	1.0 mg/L	
5 mL (vol. pipet) to 100 mL (vol. flask)	0.5 mg/L	
1 mL (vol. pipet) to 100 mL (vol. flask)	0.1 mg/L	
0.5 mL (vol. pipet) to 100 mL (vol. flask)	0.05 mg/L	
1 mL (vol. pipet) to 500 mL (vol. flask)	0.02 mg/L	

*Microbiological Sampling

Fleming Training Center | TN Department of Environment & Conservation

1

*Objectives

*Importance of proper sampling and analysis
*Selecting sampling points
*Collection, preservation, and storage of samples
*Preparation of samples for testing
*Methods for bacteriological analysis

2

*Importance of Proper Sampling

*Representative sample - contains basically the same constituents as the body of water from which it was taken

*Test results are worthless unless they indicate actual water quality

3

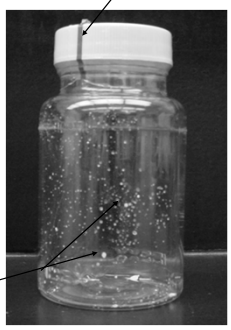
*Selecting Sampling Points

*Revised Total Coliform Rule (2016) requires written sampling plan to be approved by the State.

*Points should be representative of system:
  *Dead ends
  *Pressure zones
  *Storage facilities

*Collection of Samples

*Only approved containers should be used
  *125 mL volume
  *Pre-sterilized bottles recommended
  *Other bottles sterilized at 121ºC for 15 minutes
  *Should contain sodium thiosulfate
    *To remove chlorine



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*Collection of Samples

*Check sample bottle for flaws before use
  *Red strip indicating bottle has not been opened
  *No cracks or signs of contamination

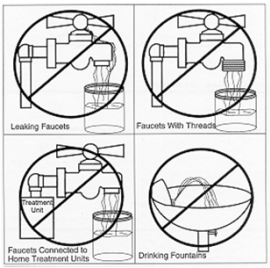
*Leave adequate space at top for mixing
  *Collect 100 mL +/- 2 mL

*Use *aseptic* technique to avoid contamination
  *Aseptic - free from contamination caused by harmful bacteria, viruses, or other microorganisms

6

*Collection of Samples

*Collect samples from approved faucets only



7

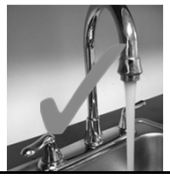
*Sample Sites cont.

*Avoid sampling from hydrants, taps from water softeners and leaking faucets
  *Very good chance for contamination
*Avoid water fountains
*Collect sample from a non-swivel faucet




*Sample Sites cont.

*Mixing faucets should not be used because water passing through the "hot" waterside may not be representative of the water in the distribution system
  *Water in the hot water tank is more likely to grow bacteria because the warm water may promote growth




*Sample Sites cont.

*Threaded taps should be avoided
  *Never use a tap with an internal thread
    *Bacteria can grow in the grooves of the threads
  *Never take samples from taps that are clearly contaminated with scum or build up around faucet


10

*Sample Sites cont.

*Avoid faucets close to the ground or sink bottom
  *Close to the ground can be contaminated with dirt
  *Close to the sink bottom may not allow room for bottle to be put under flow without touching the inside of the bottle


11

*Sample Sites cont.

*Good sample sites:
  *Water tank
  *Dead end with sampling tap
  *Fire halls
  *Public buildings


12

*Sampling Procedure cont.

*If a faucet has a strainer, aerator or any other attachment, remove before sampling

*Let the water run for several minutes (3-5 min) to insure fresh water from the main

13

*Sampling Procedure cont.

*Take Free Chlorine Residual using DPD method and record
  *Required whenever a bacteriological sample is collected
*Disinfect faucet with either a dilute bleach spray (~200 mg/L), rubbing alcohol (~25%) or flame it with propane or butane torch

14

*Sampling Procedure cont.

*Reopen the faucet and let the water run in a stream about the size of a pencil to prevent splashing during sampling
  *Remember the bottles are sterile
  *DO NOT rinse the bottle
  *If you feel you have contaminated the bottle, dispose & start with a new one
  *Use fresh bottles, old bottles left out in the sun may not eliminate chlorine as well

15

*Sampling Procedure cont.

*Hold the bottle in one hand near the bottom and with the other hand remove the top
  *Care should be taken in removing the lid so as not to touch the inside of the bottle or lid

16

*Sampling Procedure cont.

*Hold the bottle under the faucet, allowing only a small, steady stream to flow from the faucet
  *When filling the bottle, hold the bottle so as to avoid water contacting the hand and running into the bottle
  *Collect 100 mL ± 2%
    *Meniscus needs to touch the 100 mL mark
  *Immediately remove bottle from underneath the faucet and replace the lid

17

*Sampling Procedure cont.

*Document the required information from the sample site
  *Time and date collected
  *Location or address
  *Free chlorine residual
  *Collector's name
  *Sample type
    *Routine DS sample
    *Check sample
    *Raw or finished water sample
    *Repeat or confirmation sample
    *Other special purpose sample

18

*Sampling Procedure cont.

*Replace all attachments after sampling is completed

*All samples must be tested within 30 hours of collection

https://youtu.be/yyyk_M7ah3c

19

*Positive Samples

*Repeat samples shall be collected within 24 hours of positive results

*For systems that collect > 40 samples/month

*One repeat sample at original site of total coliform-positive sample

*At least one at a tap within 5 service connections upstream

*At least one at a tap within 5 service connections downstream

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*Indicator Organism

*Always present in contaminated water

*Always absent when contamination is absent

*If no indicator organism is present, then there should be no E. coli present

*Survives longer in water than other pathogens

*Is easily identified

Total Coliform Group

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*Total Coliform Bacteria

*Indicator organism – provide good index of degree of bacteriologic safety of water

*Two types

*Fecal coliform – occur in the intestines of warm-blooded animals and fecal matter

*Total coliform – consists of fecal and non-fecal coliform i.e. *Citrobacter*, *Enterbacter*, *Escherichia*, and *Klebsiella*

*Includes all the aerobic and facultative anaerobic gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose

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*Total Coliform Bacteria

*Total coliform are "presumptive" indicator of pollution

*Their presence or numbers may not necessarily relate to either the occurrence or degree of fecal pollution

*When testing for TC and EC, test must be specific and sensitive

*Incubate Total Coliform and *E. coli* samples

24 +/- 2 hrs @ 35 +/-0.5°C

23

*Total Coliform Bacteria

*Approved testing procedures

*Multiple tube fermentation method (MPN procedure)

*Presumptive test

*Confirmed test

*Completed test

*Membrane filter (MF) method

*Enzyme Substrate Tests

*aka Presence/Absence (P/A) method

24

*Multiple Tube Fermentation Method – Presumptive Test

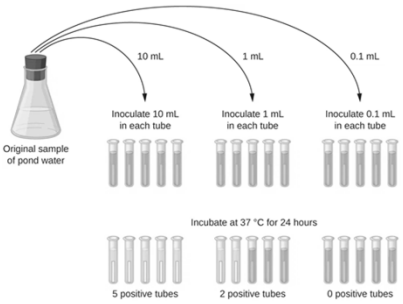
*Place portions of a water sample in lauryl tryptose broth (LTB)

*lauryl tryptose broth - standard bacteriological media containing lactose (milk) sugar and tryptose broth.

*After incubating for 24-48 hours at 35°C, examine test tubes for presence of acid and gas

25

*Multiple Tube Fermentation Method – Presumptive Test



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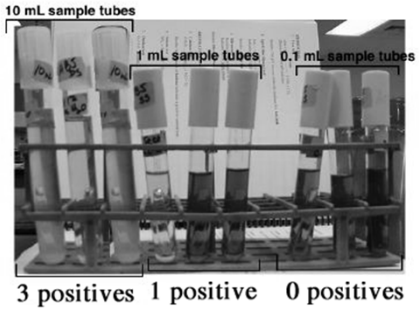
*Multiple Tube Fermentation Method - Presumptive Test



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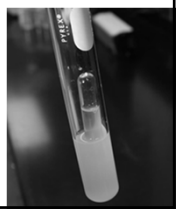
*MTF – Presumptive Test

*The presumptive test, is a screening test to sample water for the presence of coliform organisms

*If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe

*If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction

*The method of presumptive test varies for treated and untreated water



https://microbeonline.com/probable-number-mpn-test-principle-procedure-results/

*Multiple Tube Fermentation Method - Confirmed Test

*All tubes that showed color change and gas production at 24 or 48 hours must be tested for fecal coliform

*Sample from each positive tube is transferred to new test tubes with LTB and Brilliant Green Bile (BGB)

*Incubate for an additional 48 hours

*Look for gas production in sample

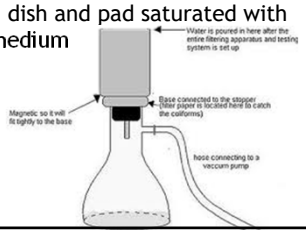
31

*Membrane Filter Method

*Filter a known volume of sample (up to 100 mL) through a sterile filter

*When the sample is filtered, bacteria (larger than 0.45 µm) are retained on the filter surface

*Filter is placed on Petri dish and pad saturated with a liquid or agar based medium



*Membrane Filter Procedure

*Prep Petri dish for filter (using aseptic technique)
- *Use wax pencil to label each petri dish
  - *Team name/number
  - *Sample (pre blank, sample 1, river, post blank, etc.)
- *Add appropriate liquid medium to sample
  - *Total coliform – m-Endo
  - *E. coli – m-coliblue
  - *Decant excess medium
- *Replace lid on petri dish

33

*Membrane Filter Procedure

*Set up vacuum pump, hose, vacuum funnel
*Obtain sterile filter apparatus (2 pieces), sterile filters, alcohol burner, tweezers, sample(s), beaker with alcohol
*Sterilize forceps by dipping in alcohol and passing quickly through flame to burn off alcohol
*Using aseptic technique, place filter on filter apparatus (grid side up)
*Replace funnel top

34

*Membrane Filter Procedure

*Pour appropriate sample volume into funnel
- *If using small sample dilution, add dilution water to sample prior to starting vacuum
- *Apply vacuum

*Rinse filter with three 20 to 30 mL portions of sterile dilution water
*Resterilize forceps, remove filter from funnel
*Center membrane filter on pad containing m-Endo media with a rolling motion to ensure water seal
- *If portions of membrane do not have m-endo stain, there is an air bubble that must be removed

35

*Membrane Filter Procedure

*Invert dish and incubate
- *24 hours at 35 +/- 0.5°C

*Using low power microscope, count colonies formed
- *Total Coliform – red with green-gold metallic sheen
  - *When using m-Endo
- *E. coli – Blue
  - *When using m-coliblue

*Report number of colonies as CFUs (colony forming units)

*Expected Reactions of Various Microorganisms

*Total coliforms will produce a red colony
- *_Enterobacter species_
  - *_E. cloacae_
  - *_E. aerogenes_
- *_Klebsiella species_
  - *_K. pneumoniae_
- *_Citrobacter species_
  - *_C. freundii_

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*Expected Reactions of Various Microorganisms

*_Escherichia coli_ will produce a blue colony
- *_E. coli_ O157:H7 will not produce a blue colony, but will grow as a red colony

*Known negative reaction (no growth) after 24-25 hours
- *_Pseudomonas aeruginosa_
  - *Variable reaction may be positive for total coliform when incubated longer than 25 hours
- *_Proteus vulgaris_
- *_Aeromonas hydrophila_

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*Membrane Filter Procedure

Bacteria	Medium	Temperature	Time	Results
Total Coliform	m-Endo	35 +/- 0.5°C	22-24 hours	Red colony with green-gold metallic sheen
Fecal Coliform (heat tolerant bacteria)	m-FC	44.5 +/- 0.2°C	22-24 hours	Blue colony
E. coli	m-coliblue	35 +/- 0.5°C	22-24 hours	Blue colony

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*Enzyme Substrate Tests

- *Use a media that contains specific indicator nutrients for TC and EC
  - *As nutrients are metabolized, yellow color and fluorescence are released confirming the presence of TC and EC
- *Non-coliform bacteria are suppressed to prevent interference
- *Two common enzyme substrate tests
  - *Colilert
  - *Colisure

40

*Presence/Absence Method

- *One large portion in a single bottle is used to obtain qualitative information on the presence or absence of coliforms
  - *Media placed in sample during incubation time is a mixture of lactose and lauryl tryptose broths with bromcresol purple
  - *Coliforms are present if gas or acid (yellow colored) is produced



41

*Presence/Absence Method

- *Colilert (P/A)
- *Colilert Quanti-Tray
- *Colilert-18 (P/A)
- *Colilert-18 Quanti-Tray
- *E*Colite
- *Colisure
- *Readycult Coliforms 100 (P/A) and Fluorocult LMX Broth
- *Colitag

Incubate Total Coliform and E. coli samples
24 +/- 2 hrs @ 35 +/-0.5°C

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*Colilert's "Blue Flash"

- *Samples containing excessive amounts of free chlorine can cause this reaction
- *A 700 mg/l level of chlorine will produce this transient blue color when Colilert is added
- *Sometimes gas production can be seen immediately after the Colilert is added along with the blue reaction
- *The sample should be considered invalid and it is recommended to resample and retest
- *The source and/or amount of chlorine in the sample should also be investigated for 700 mg/l is not a typical chlorine level in a water sample

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*Colilert's "Blue Flash"

- *This can be done if someone has poured bleach into a well or even "dosed" a bac't bottle with as little as 1.3 mL of fresh bleach.
- *To catch lower doses of chlorine for outside samples, send two bottles, one with sodium thiosulfate for your bac't analysis and one without to check for chlorine
  - *There should not be any chlorine present in a well sample

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Glossary of Microbiological Terms

Aseptic – free from germs, fermentation, or putrefaction; sterile.

Aseptic technique – procedure in which no microbiological contamination occurs.

Autoclave – device used for sterilizing laboratory equipment by using pressurized steam.

Coliform bacteria – a group of bacteria including all the aerobic and facultative anaerobic gram-negative, non-spore forming, rod shaped bacteria that ferment lactose with gas and acid production within 48 hours at 35°C. The presence of coliform bacteria in drinking water indicates fecal contamination.

Colilert Method – a presence/absence test for total coliforms that will also confirm the presence or absence of *E. coli* within 24 hours.

Composite sample – series of samples taken at different times and mixed together to determine average concentration of a substance in the water.

Desiccator – heavy glass container that allows substances to be cooled in a dry environment. It is used to store substances that readily absorb moisture from the air. It contains a chemical in the bottom that absorbs moisture and keeps the relative humidity near zero.

Dilution water – reagent grade water that is buffered with potassium dihydrogen phosphate and magnesium chloride and sterilized in an autoclave. It is used for rinsing the funnel used in the membrane filter method, and for diluting samples.

Escherichia coli (*E. coli*) – a bacteria of the fecal coliform group. Its presence may be tested for in lieu of fecal coliforms following a total coliform positive sample in the distribution system. This organism is used for quality control of m-Endo broth for bacteriological testing.

Fecal coliform – a bacteria of the coliform group that indicates fecal contamination. The presence of fecal coliforms in a water sample is a violation of the Total Coliform Rule.

Grab sample – a sample of water collected at a specific time and place that represents water quality at that time and place.

Graduated cylinder – glass or plastic container used for making measurements of volume. They are less accurate than pipets and volumetric flasks, but are more accurate than beakers and Erlenmeyer flasks.

Heterotrophic Plate Count (HPC) – laboratory procedure used for estimating the total bacterial count in a water sample. Also called standard plate count.

Incubator – heated container that maintains a constant temperature for the proper development of microbiological cultures.

Indicator organism – an organism that is always present in contaminated water, is not present in uncontaminated water, survives longer in water than other pathogens, and is easily identified. The indicator organism for bacteriological quality of drinking water is the total coliform group.

Klebsiella – a member of the coliform group, used for quality control of media for bacteriological testing.

m-Endo broth – nutritive media used in the membrane filter method for total coliform analysis.

Maximum Contaminant Level (MCL) – the maximum permissible level of a contaminant in drinking water as specified by the Safe Drinking Water Act.

Membrane Filter Method – test for coliform bacteria that uses a 0.45µ filter to trap coliform bacteria from a 100 mL sample, then incubates the bacteria in a petri dish with nutritive broth at 35°C for 24 hours. The presence of total coliforms is indicated by dark colonies with a green-gold metallic sheen.

MPN – Most Probable Number of coliform bacteria in a 100 mL sample. Used in the Multiple Tube Fermentation Method and other methods

Pathogen – an organism capable of causing disease in a host.

Petri dish – a round, shallow glass or plastic dish with a loose-fitting lid used for bacterial cultures.

Pipet – glass tube used for accurately measuring small volumes. Includes volumetric, Mohr, and serological.

Potable water – water that does not contain objectionable pollution, contamination, minerals, or infective agents, and is considered satisfactory for drinking

Representative sample – sample that contains basically the same constituents as the body of water from which it was taken.

Sodium thiosulfate – chemical in a bacteriological sample bottle that neutralizes the chlorine residual in a water sample.

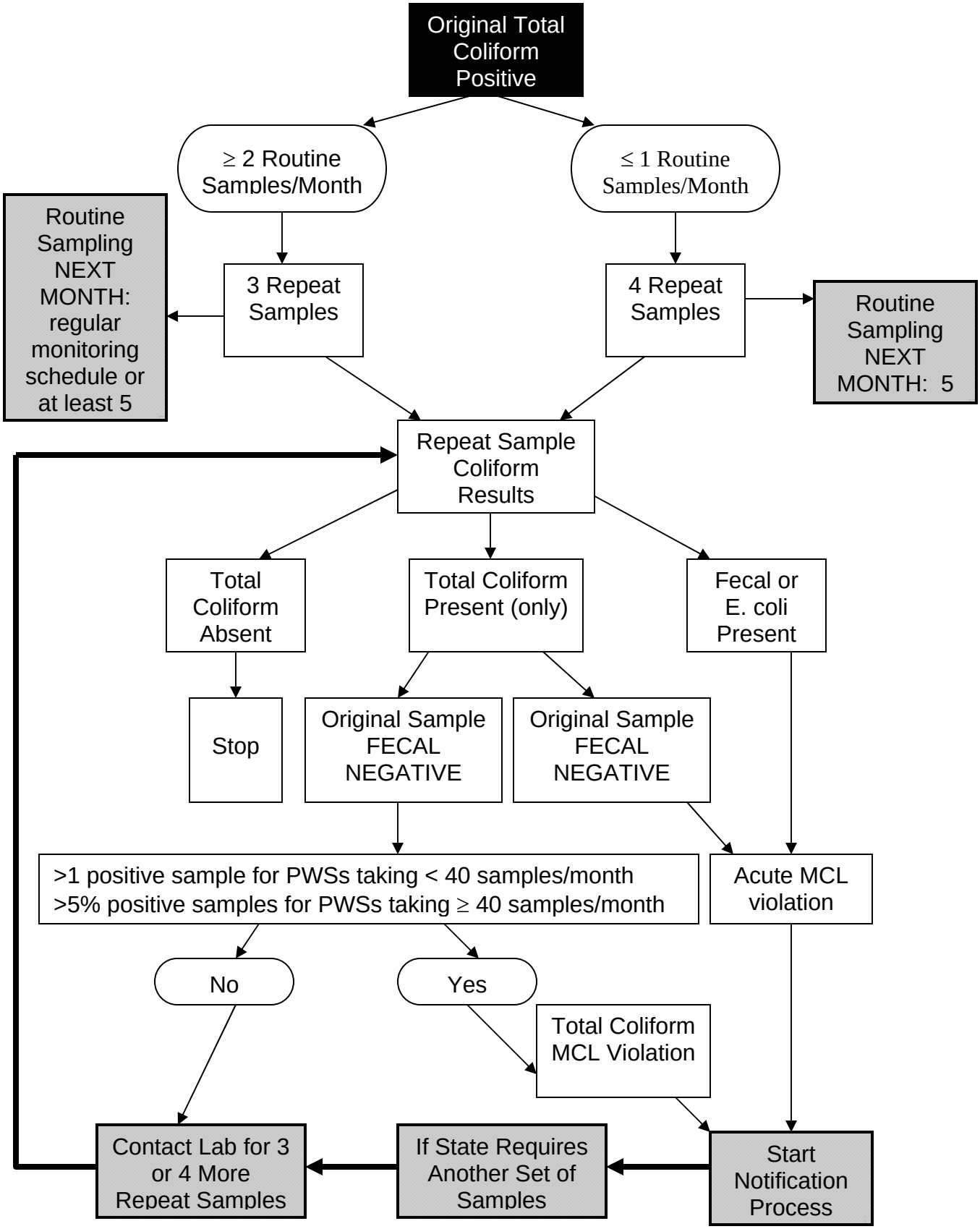
Sterilization – destruction or inactivation of all microorganisms.

Total Coliform Rule – a regulation that became effective December 31, 1990. It changed the MCL for total coliforms from a quantitative density to presence/absence.

Volumetric flask – glassware used for mixing accurate solutions. Each flask is calibrated for a single volume.

Waterborne disease – an illness that is caused a pathogenic organism carried by water.

Total Coliform Action Flow Chart



9050 PREPARATION OF CULTURE MEDIA*

9050 A. General Procedures

1. Storage of Culture Media

Store dehydrated media (powders) in tightly closed bottles in the dark at less than 30°C in an atmosphere of low humidity, e.g., desiccator. Do not use them if they are discolored or caked, or if the character of a free-flowing powder is lost. Use stocks of dehydrated media before their expiration date or use within a year of purchase if dehydrated media contain selective agents, such as sodium azide, bile salts, antibiotics, sulfur-containing amino acids, etc., so as to maintain optimum selectivity. Review manufacturer's instructions, product Material Safety Data Sheets (MSDS), and analytical methods before preparing media. Use commercially prepared media wherever available. Avoid preparing media from essential ingredients unless necessary. See also Section 9020B.5*j*.

Prepare culture media in batches that will be used in less than 2 weeks unless otherwise specified by the method. However, if the media are contained in screw-capped tubes they may be stored for up to 3 months. See Table 9020:V for specific details. Store media out of direct sunlight and avoid excessive evaporation. Place prepared petri dishes in airtight containers or plastic bags, close with twist-ties, and store under refrigerated conditions. Invert petri dishes to prevent moisture condensation on agar.

If refrigerated, liquid media in fermentation tubes may dissolve sufficient air to produce, upon incubation at 35°C, a bubble of air in the tube. Bring all media (especially fermentation or carbohydrate broth) to room temperature before use and discard tubes containing air bubble.

Fermentation tubes may be stored at approximately 25°C; but because evaporation may proceed rapidly under these conditions—resulting in marked changes in concentration of the ingredients—do not store at this temperature for more than 2 weeks. Discard tubes with growth due to contamination or an evaporation loss of more than 1 mL. A 1-mL or greater loss from the initial 10 mL can affect most-probable-number calculations. Selective agents also may break down after prolonged incubation or storage.

2. pH Adjustment

Determine and record pH of medium after sterilization. The required final pH is given in the directions for preparing each medium. If a specific pH is not prescribed, adjustment is unnecessary. The decrease in pH during sterilization will vary slightly with the individual sterilizer in use, and the initial pH required to obtain the correct final reaction will have to be determined. The decrease in pH usually will be 0.1 to 0.2 but occasionally may be as great as 0.3 in double-strength media. When buffers are present in the media the decrease in pH value will be negligible.

For determination of final pH, cool medium to 44 to 46°C, aseptically remove a small quantity, and determine pH after setting the meter for the higher temperature if not done automatically. Alternatively, cool a sample of the medium completely to room temperature before determining final pH. If pH adjustment is necessary, use sterile stir bar and pipet a sufficient quantity of filter-sterilized 0.1*N* NaOH or 0.1*N* HCl into the bulk medium to reach the proper pH.

The pH of reconstituted dehydrated media seldom will require adjustment if made according to directions. Errors in weighing dehydrated medium, overheating reconstituted medium, or a problem with the medium itself may produce an unacceptable final pH. If pH values of prepared media are consistently outside the allowed pH range, determine cause. Adjustment of pH before sterilization may be necessary.

3. Sterilization

After rehydrating a medium, dispense promptly to culture vessels and sterilize within 2 h. Do not store nonsterile media.

Sterilize media in an autoclave at 121°C. The required exposure time will vary with form and type of material, medium, presence of carbohydrates, and volume. For example, sterilize carbohydrate broths at 121°C for only 12 to 15 min. after the temperature has reached 121°C. When the pressure reaches zero, remove medium from autoclave and cool quickly to avoid decomposition of sugars by prolonged exposure to heat. To permit uniform heating and rapid cooling, pack materials loosely and in small containers. The maximum elapsed time for exposure of carbohydrate broths to any heat (from time of closing loaded autoclave to unloading) is 45 min. Preferably use a double-walled autoclave to permit preheating before loading to reduce total heating time to within the 45-min or less limit. Adjust autoclave times as volumes/loads increase. Presterilized media may be available commercially.

4. Quality Control

See Section 9020B.5*j*.

5. Bibliography

BUNKER, G.C. & H. SCHUBER. 1922. The reaction of culture media. *J. Amer. Water Works Assoc.* 9:63.

BALOWS, A., W.J. HAUSLER, JR., K.L. HERRMANN, H.D. ISENBERG & H.J. SHADOMY, eds. 1991. Manual of Clinical Microbiology, 5th ed. American Soc. Microbiology, Washington, D.C.

RICHARDSON, G.H., ed. 2004. Standard Methods for the Examination of Dairy Products, 17th ed. American Public Health Assoc., Washington, D.C.

* Approved by Standard Methods Committee, 2006.
Joint Task Group: Margo E. Hunt (chair), Ellen B. Braun-Howland, Terry C. Covert, Gil Dichter, Nancy H. Hall, Robin K. Oshiro.

9050 B. Water

1. Specifications

To prepare culture media and reagents, use only distilled or demineralized reagent-grade water that has been tested and found free from traces of dissolved metals and bactericidal or inhibitory compounds. Toxicity in distilled water may be derived from fluoridated water high in silica. Other sources of toxicity are silver, lead, and various unidentified organic complexes. Where condensate return is used as feed for a still, toxic amines or other boiler compounds may be present in distilled water. Residual chlorine or chloramines also may be found in distilled water prepared from chlorinated water supplies. If chlorine compounds are found in distilled water, neutralize them by adding an equivalent amount of sodium thiosulfate or sodium sulfite.

Distilled water also should be free of contaminating nutrients. Such contamination may be derived from flashover of organics during distillation, continued use of exhausted carbon filter beds, deionizing columns in need of recharging, solder flux residues in new piping, dust and chemical fumes, and storage of water in unclean bottles.

Store distilled water out of direct sunlight to prevent growth of algae. Aged distilled water may contain toxic volatile organic compounds absorbed from the atmosphere if stored for prolonged periods in unsealed containers. Good housekeeping practices that minimize the presence of airborne particulates usually will eliminate nutrient contamination.

See Section 9020B and Table 9020:II.

2. Bibliography

STRAKA, R.P. & J.L. STOKES. 1957. Rapid destruction of bacteria in commonly used diluents and its elimination. *Appl. Microbiol.* 5:21.

GELDREICH, E.E. & H.F. CLARK. 1965. Distilled water suitability for microbiological applications. *J. Milk Food Technol.* 28:351.

MACLEOD, R.A., S.C. KUO & R. GELINAS. 1967. Metabolic injury to bacteria. II. Metabolic injury induced by distilled water or Cu^{++} in the plating diluent. *J. Bacteriol.* 93:961.

9050 C. Media Specifications

The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable commercially prepared dehydrated media are available. Follow manufacturer's directions for rehydration and sterilization. Commercially prepared media in liquid form (sterile ampule or other) also may be used if known to give equivalent results. See Section 9020B.5*j* for quality-control specifications.

The terms used for protein source in most media, for example, peptone, tryptone, tryptose, were coined by the developers of the media and may reflect commercial products rather than clearly defined entities. It is not intended to preclude the use of alternative materials provided that they produce equivalent results.

NOTE: The term *percent solution* as used in these directions is to be understood to mean "grams of solute per 100 mL solution."

1. Dilution Water

Various dilution water solutions can be prepared. The following represent two of the most commonly used solutions in the basic water microbiology laboratory.

a. Buffered water:

1) Stock phosphate buffer solution—Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL reagent-grade water, adjust to pH 7.2 ± 0.5 with 1*N* sodium hydroxide (NaOH), and dilute to 1 L with reagent-grade water. Sterilize by filtration or autoclave. Store stock solution under refrigerated conditions and discard if turbidity develops.

2) Magnesium chloride stock solution—Add magnesium chloride (38 g/L $MgCl_2$ or 81.1 g $MgCl_2 \cdot 6H_2O$) to 1 L reagent-grade water. Sterilize and store stock solution under refrigerated conditions, discarding if solution becomes turbid.

3) Working solution—Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride stock solution to 1 L reagent-grade water. Dispense in amounts that will provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min. Final pH should be 7.2 ± 0.1. Note that pH values will change with time. Store under refrigerated conditions after opening and discard if turbidity develops. Use within 6 months.

b. Peptone water, 0.1%: Prepare by adding 1 g peptone to 1 L reagent water. Final pH should be 7.0 ± 0.2 after sterilization.

Dispense in amounts to provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min. Store as above.

Do not suspend a sample in any dilution water for more than 30 min at room temperature because injury, death, or multiplication may occur.

2. Culture Media

Specifications for individual media are included in subsequent sections. Details are provided where use of a medium is first described.

3. Bibliography

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA-600/8-78-017, U.S. Environmental Protection Agency, Cincinnati, Ohio.

9221 MULTIPLE-TUBE FERMENTATION TECHNIQUE FOR MEMBERS OF THE COLIFORM GROUP*

9221 A. Introduction

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of this group has been based on the method used for detection, lactose fermentation, rather than on the tenets of systematic bacteriology. Accordingly, when the fermentation technique is used, this group is defined as all facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35°C.

The standard test for the coliform group may be carried out by the multiple-tube fermentation technique or presence–absence procedure (through the presumptive-confirmed phases or completed test) described herein, the membrane filter (MF) technique (Section 9222) or the enzymatic substrate coliform test (Section 9223). Each technique is applicable within the limitations specified and with due consideration of the purpose of the examination. Production of valid results requires strict adherence to quality control procedures. Quality control guidelines are outlined in Section 9020.

When multiple tubes are used in the fermentation technique, coliform density can be estimated by using a most probable number (MPN) table. This number, based on certain probability formulas, is an estimate of the mean density of coliforms in the sample. Results of coliform testing, together with other information obtained from engineering or sanitary surveys, provide the best assessment of water treatment effectiveness and the sanitary quality of source water.

The precision of the fermentation test in estimating coliform density depends on the number of tubes used. The most satisfactory information will be obtained when the largest sample inoculum examined shows acid and/or gas in some or all of the tubes and the smallest sample inoculum shows no acid and/or gas in any or a majority of the tubes. Bacterial density can be estimated by the formula given or from the table using the number of positive tubes in the multiple dilutions (9221C.2). The number of sample portions selected will be governed by the desired precision of the result. The MPN tables are based on the assumption of a Poisson distribution (random dispersion). However, if the sample is not adequately shaken before the portions are removed or if bacterial cells clump, the MPN value will be an underestimate of actual bacterial density.

1. Water of Drinking Water Quality

When analyzing drinking water to determine if the quality meets U.S. Environmental Protection Agency (EPA) standards, use the fermentation technique with 10 replicate tubes each containing 10 mL, 5 replicate tubes each containing 20 mL, or a single bottle containing a 100-mL sample portion. When examining drinking water via the fermentation technique, process all tubes or bottles demonstrating growth, with or without a positive acid or gas reaction, to the confirmed phase (9221B.3). Drinking water samples that are positive for total coliforms also must be tested for thermotolerant (fecal) coliforms (9221E) or *Escherichia coli* (9221F).

For routine examinations of public water supplies, the objective of the total coliform test is to determine the efficiency of treatment plant operations and the integrity of the distribution system. It is also used as a screen for the presence of fecal contamination. Some coliform occurrences in a distribution system may be attributed to growth or survival of coliforms in bacterial biofilms in the mains, rather than treatment failure at the plant or well source, or outside contamination of the distribution system. Because it is difficult to distinguish between coliforms entering the distribution system and coliforms already present in the pipe biofilm and sediments, assume that all coliforms originate from a source outside the distribution system.

2. Water of Other than Drinking Water Quality

When examining nonpotable waters, inoculate a series of tubes with appropriate decimal dilutions of the water (multiples of 10 mL), based on the probable coliform density. Use the presumptive–confirmed phases of the multiple-tube procedure. Use the more labor-intensive completed test (9221B.4) as a quality control measure on at least 10% of coliform-positive nonpotable water samples on a seasonal basis. The objective of the examination of nonpotable water, generally, is to estimate the bacterial density, determine a source of pollution, enforce water quality standards, or trace the survival of microorganisms. The multiple-tube fermentation technique may be used to obtain statistically valid MPN estimates of coliform density. Examine a sufficient number of water samples to yield representative results for the sampling station. Generally, the geometric mean or median value of the results of a number of samples will yield a value in which the effect of sample-to-sample variation is minimized.

3. Other Samples

The multiple-tube fermentation technique is applicable to the analysis of salt or brackish waters as well as muds, sediments, and sludges. Collect samples as directed in Sections 9060A and B, using sample containers specified in Section 9030B.19. Follow the precautions given above on portion sizes and numbers of tubes per dilution.

* Approved by Standard Methods Committee, 2006.
Joint Task Group: Ellen B. Braun-Howland (chair), Paul S. Berger, Robert J. Blodgett, Clifford H. Johnson, Shundar Lin, Mark C. Meckes, Eugene W. Rice.

To prepare solid or semisolid samples, weigh the sample and add diluent to make a 10^{-1} dilution. For example, place 30 g sample in sterile blender jar, add 270 mL sterile phosphate buffered or 0.1% peptone dilution water, and blend for 1 to 2 min at high speed (8000 rpm). Prepare the appropriate decimal dilutions of the homogenized slurry as quickly as possible to minimize settling.

9221 B. Standard Total Coliform Fermentation Technique

1. Samples

Collect samples as directed in Sections 9060A and B, using sample containers specified in Section 9030B.19.

2. Presumptive Phase

Use lauryl tryptose broth in the presumptive portion of the multiple-tube test. If the medium has been refrigerated after sterilization, incubate overnight at room temperature (20°C) before use. Discard tubes showing growth and/or bubbles.

a. Reagents and culture medium: If possible, use a commercially available medium.

Lauryl tryptose broth:

Tryptose	20.0	g
Lactose	5.0	g
Dipotassium hydrogen phosphate, K_2HPO_4	2.75	g
Potassium dihydrogen phosphate, KH_2PO_4	2.75	g
Sodium chloride, NaCl	5.0	g
Sodium lauryl sulfate	0.1	g
Reagent-grade water	1	L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. Before sterilization, dispense—in fermentation tubes with an inverted vial (Durham tube)—sufficient medium to cover the inverted vial at least one-half to two-thirds after sterilization. Alternatively, omit the inverted vial and add 0.01 g/L bromcresol purple to lauryl tryptose broth to determine acid production, an indicator of a positive result in this part of the coliform test. Close tubes with metal or heat-resistant plastic caps.

Make lauryl tryptose broth of such strength that adding 100-mL, 20-mL, or 10-mL portions of sample to the medium will not reduce ingredient concentrations below those of the standard medium. Prepare in accordance with Table 9221:I. Autoclave medium at 121°C for 12 to 15 min. Ensure that inverted vials, if used, are free of air bubbles. Medium pH should be 6.8 ± 0.2 after sterilization.

TABLE 9221:I. PREPARATION OF LAURYL TRYPTOSE BROTH

Inoculum *mL*	Amount of Medium in Tube *mL*	Volume of Medium + Inoculum *mL*	Dehydrated Lauryl Tryptose Broth Required *g/L*
1	10 or more	11 or more	35.6
10	10	20	71.2
10	20	30	53.4
20	10	30	106.8
100	50	150	106.8
100	35	135	137.1
100	20	120	213.6

b. Procedure:

1) Arrange fermentation tubes in rows of five or ten tubes each in a test tube rack. The number of rows and the sample volumes selected depend on the quality and character of the water to be examined. For potable water, use five 20-mL portions, ten 10-mL portions, or a single bottle of 100-mL portion; for nonpotable water, use five tubes per dilution (of 10, 1, 0.1 mL, etc.).

When making dilutions and measuring diluted sample volumes, follow the precautions given in Section 9215B.2. Use Figure 9215:1 as a guide to preparing dilutions. Shake sample and dilutions vigorously about 25 times. Inoculate each tube in a set of five with replicate sample volumes in increasing decimal dilutions, if decimal quantities of the sample are used. Mix test portions in the medium by gentle agitation.

2) Incubate inoculated tubes or bottles at 35 ± 0.5°C. After 24 ± 2 h swirl each tube or bottle gently and examine it for growth, gas, and/or acidic reaction (shades of yellow color) and, if no gas or acidic reaction is evident, re-incubate and re-examine at the end of 48 ± 3 h. Record presence or absence of growth, gas, and/or acid production. If the inner vial is omitted, growth with acidity (yellow color) signifies a positive presumptive reaction.

c. Interpretation: Production of an acidic reaction and/or gas in the tubes or bottles within 48 ± 3 h constitutes a positive presumptive reaction. Submit tubes or bottles with a positive presumptive reaction to the confirmed phase (9221B.3).

The absence of acidic reaction and/or gas formation at the end of 48 ± 3 h of incubation constitutes a negative test. Submit drinking water samples demonstrating growth without a positive gas or acid reaction to the confirmed phase (9221B.3). An arbitrary 48-h limit for observation doubtless excludes occasional members of the coliform group that grow very slowly (see Section 9212).

3. Confirmed Phase

a. Culture medium: Use brilliant green lactose bile broth fermentation tubes for the confirmed phase. If possible, use a commercially available medium.

Brilliant green lactose bile broth:

Peptone	10.0	g
Lactose	10.0	g
Oxgall	20.0	g
Brilliant green	0.0133	g
Reagent-grade water	1	L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. Before sterilization, dispense—in fermentation tubes with an inverted vial—sufficient medium to cover the inverted vial at least one-half to two-thirds after sterilization. Close tubes

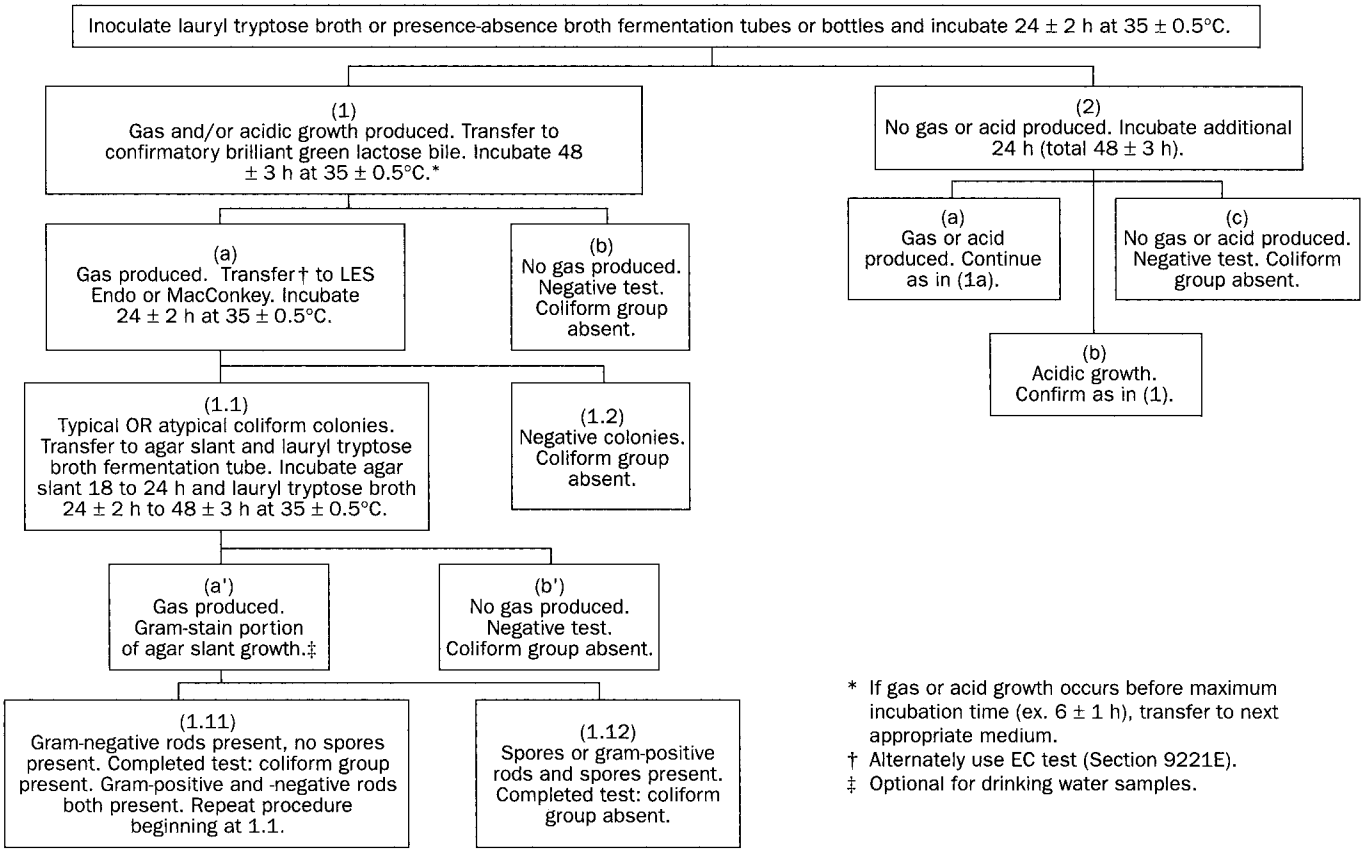


Figure 9221:1. Schematic outline of presumptive, confirmed, and completed phases for total coliform detection.

with metal or heat-resistant plastic caps. Autoclave medium at 121°C for 12 to 15 min. Ensure that inverted vials are free of air bubbles. Medium pH should be 7.2 ± 0.2 after sterilization.

b. Procedure: Submit all presumptive tubes or bottles showing growth, any amount of gas, or acidic reaction within 24 ± 2 h of incubation to the confirmed phase. If additional presumptive tubes or bottles show active fermentation or acidic reaction at the end of a 48 ± 3 h incubation period, submit these to the confirmed phase. Simultaneous inoculation into brilliant green lactose bile broth for total coliforms and EC broth for thermotolerant (fecal) coliforms (see 9221E) or EC-MUG broth for *Escherichia coli* (see 9221F) may be used. To confirm presumptive coliform colonies growing on a solid medium using fermentation media, see Section 9222B.4*f*.

Gently shake or rotate presumptive tubes or bottles showing gas or acidic growth to resuspend the organisms. With a sterile loop 3.0 to 3.5 mm in diameter, transfer one or more loopfuls of culture to a fermentation tube containing brilliant green lactose bile broth. Alternatively, insert a sterile wooden applicator at least 2.5 cm into the culture, promptly remove, and plunge applicator to bottom of fermentation tube containing brilliant green lactose bile broth. Remove and discard applicator. Repeat for all other positive presumptive tubes.

Incubate the inoculated brilliant green lactose bile broth tubes at 35 ± 0.5°C. Formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube at any time within 48 ± 3 h constitutes a positive confirmed phase. To estimate the coliform density, calculate the MPN value from the number of positive brilliant green lactose bile tubes (as described in 9221C).

c. Alternative procedure: Use this alternative only for polluted water or wastewater known to produce positive results consistently.

If all presumptive tubes are positive in two or more consecutive dilutions within 24 h, submit to the confirmed phase only the tubes of the highest dilution (smallest sample inoculum) in which all tubes are positive and any positive tubes in still higher dilutions. Submit to the confirmed phase all tubes in which gas or acidic growth is produced in 24 to 48 h.

4. Completed Phase

To verify the presence of coliform bacteria and to provide quality control data for nonpotable water sample analysis, use the completed test on 10% (or a set percentage) of positive confirmed tubes (see Figure 9221:1). Use the completed test on at least one positive sample per quarter. If no positive sample occurs within a quarter, a QC check must be performed using a known positive sample. Simultaneous inoculation into brilliant green lactose bile broth for total coliforms and EC broth for thermotolerant (fecal) coliforms (9221E) or EC MUG broth for *Escherichia coli* (9221F) may be used. Positive results from incubation in EC and/or EC-MUG broths at elevated temperature (44.5 ± 0.2°C) can be considered as a completed test. Parallel

positive brilliant green lactose bile broth cultures with negative EC or EC-MUG broth cultures indicate the presence of nonfecal coliforms. Parallel positive EC or EC-MUG tubes and negative brilliant green lactose bile broth cultures are recorded as positive for fecal coliforms or *E. coli*, respectively. Alternatively, the completed test for positive total coliforms may be performed as follows.

a. Culture media and reagents: If possible, use a dehydrated, commercially available medium.

1) *LES Endo agar:* See Section 9222B.2*a*. Use 100- × 15-mm petri plates.

2) *MacConkey agar:*

Peptone	17	g
Proteose peptone	3	g
Lactose	10	g
Bile salts	1.5	g
Sodium chloride, NaCl	5	g
Agar	13.5	g
Neutral red	0.03	g
Crystal violet	0.001	g
Reagent-grade water	1	L

Add ingredients to water, mix thoroughly, and heat to boiling to dissolve. Sterilize by autoclaving for 15 min at 121°C. Temper agar after sterilization and pour into petri plates (100 × 15 mm). Medium pH should be 7.1 ± 0.2 after sterilization.

3) *Nutrient agar:*

Peptone	5.0	g
Beef extract	3.0	g
Agar	15.0	g
Reagent-grade water	1	L

Add ingredients to water, mix thoroughly, and heat to dissolve. Before sterilization, dispense in screw-capped tubes. Autoclave at 121°C for 15 min. Medium pH should be 6.8 ± 0.2 after sterilization. After sterilization, immediately place tubes in an inclined position so the agar will solidify with a sloped surface. Tighten screw caps after cooling and store in a protected, cool storage area.

4) *Gram-stain reagents:* Reagents are commercially available as prepared solutions.

a) *Ammonium oxalate-crystal violet (Hucker's):* Dissolve 2 g crystal violet (90% dye content) in 20 mL 95% ethyl alcohol. Dissolve 0.8 g $(NH_4)_2C_2O_4 \cdot H_2O$ in 80 mL reagent-grade water. Mix the two solutions and age for 24 h before use. Filter through paper into a staining bottle.

b) *Lugol's solution, Gram's modification:* Grind 1 g iodine crystals and 2 g KI in a mortar. Add reagent-grade water a few milliliters at a time, and grind thoroughly after each addition until solution is complete. Rinse solution into an amber glass bottle with the remaining water, using a total of 300 mL.

c) *Counterstain:* Dissolve 2.5 g safranin dye in 100 mL 95% ethyl alcohol. Add 10 mL to 100 mL reagent-grade water. **CAUTION: Flammable.**

d) *Acetone alcohol:* Mix equal volumes of ethyl alcohol (95%) with acetone. **CAUTION: Flammable.**

b. Procedure:

1) Using aseptic technique, streak one LES Endo agar (Section 9222B.2*a*.) or MacConkey agar plate from each tube of brilliant green lactose bile broth showing gas, as soon as possible after the observation of gas. Streak plates in a manner to ensure the presence of some discrete colonies separated by at least 0.5 cm. To obtain a high proportion of successful isolations if coliform organisms are present, the following approach may be used: (*a*) Use a sterile 3-mm-diam loop or an inoculating needle slightly curved at the tip; (*b*) tap and incline the fermentation tube to avoid picking up any membrane or scum on the needle; (*c*) insert the end of the loop or needle into the liquid in the tube to a depth of approximately 0.5 cm; and (*d*) streak a plate for isolation with the curved section of the needle in contact with the agar to avoid a scratched or torn surface. Flame the loop between the second and third quadrants to improve colony isolation.

Incubate plates, inverted, at 35 ± 0.5°C for 24 ± 2 h.

2) The colonies developing on LES Endo agar are defined as *typical* (pink to dark red with a green metallic surface sheen) or *atypical* (pink, red, white, or colorless colonies without sheen) after 24 h incubation. Typical lactose-fermenting colonies developing on MacConkey agar are red and may be surrounded by an opaque zone of precipitated bile. From each plate pick one or more typical, well-isolated coliform colonies or, if no typical colonies are present, pick two or more colonies considered most likely to be organisms of the coliform group. Transfer growth from each isolate to a single-strength lauryl tryptose broth fermentation tube and onto a nutrient agar slant.

If needed, use a colony magnifying device to provide optimum magnification when colonies are picked from the LES Endo or MacConkey agar plates. When transferring colonies, choose well-isolated ones and barely touch the surface of the colony with a flame-sterilized, air-cooled transfer needle to minimize the danger of transferring a mixed culture.

Incubate secondary broth tubes (lauryl tryptose broth with inverted fermentation vials) at 35 ± 0.5°C for 24 ± 2 h; if gas is not produced within 24 ± 2 h, reincubate and examine again at 48 ± 3 h. Microscopically examine Gram-stained preparations from those 24-h nutrient agar slant cultures corresponding to the secondary tubes that show gas.

3) Gram-stain technique—The Gram stain may be omitted from the completed test for potable water samples only because the occurrences of gram-positive bacteria and spore-forming organisms surviving this selective screening procedure are infrequent in drinking water.

Various modifications of the Gram stain technique exist. Use the following modification by Hucker for staining smears of pure cultures; include a gram-positive and a gram-negative culture as controls.

Prepare separate light emulsions of the test bacterial growth and positive and negative control cultures on the same slide, using drops of distilled water on the slide. Air-dry, fix by passing slide through a flame, and stain for 1 min with ammonium oxalate-crystal violet solution. Rinse slide in tap water, and drain off excess; apply Lugol's solution for 1 min.

Rinse stained slide in tap water. Decolorize for approximately 15 to 30 s with acetone alcohol by holding slide between the fingers and letting acetone alcohol flow across the stained smear until the solvent flows colorlessly from the slide. Do not overdecolorize. Counterstain with safranin for 15 s, rinse with tap water, blot dry with absorbent paper or air dry, and examine microscopically. Gram-positive organisms are blue; gram-negative organisms are red. Results are acceptable only when controls have given proper reactions.

c. Interpretation: Formation of gas in the secondary tube of lauryl tryptose broth within 48 ± 3 h and demonstration of gram-negative, nonspore-forming, rod-shaped bacteria from the agar culture constitute a positive result for the completed test, demonstrating the presence of a member of the coliform group.

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9221 C. Estimation of Bacterial Density

1. Precision of the Multiple-Tube Fermentation Test

Unless many sample portions are examined, the precision of the Multiple-Tube Fermentation Test is rather low. Consequently, use caution when interpreting the sanitary significance of any single coliform result. When several samples from a given sampling point are estimated separately and the results combined in their geometric mean, the precision is greatly improved. Although most probable number (MPN) tables and calculations are described for use in the coliform test, they also can be used to determine the MPN of any other organisms, provided that suitable test media are available.

2. Table Reading and Recording of Most Probable Number (MPN)

Record coliform concentration as MPN/100 mL. MPN values for a variety of positive and negative tube combinations are given in Tables 9221:II, III, and IV. The sample volumes indicated in Tables 9221:II and III are chosen especially for examining drinking waters. Table 9221:IV illustrates MPN values for combinations of positive and negative results when five 10-mL, five 1.0-mL, and five 0.1-mL sample portion volumes of nonpotable water are tested. If the sample portion volumes used are those found in the tables, report the value corresponding to the number of positive and negative results in the series as MPN/100 mL. When the series of decimal dilutions is different from that in Table 9221:IV, select the MPN value from Table 9221:IV for the combination of positive results and calculate according to the following formula:

$$\text{MPN/100 mL} = (\text{Table MPN/100 mL}) \times 10/V$$

TABLE 9221:II. MPN INDEX AND 95% CONFIDENCE LIMITS FOR ALL COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 20-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 5 (20 mL Each)	MPN Index/ 100 mL	95% Confidence Limits (Exact)	
		Lower	Upper
0	<1.1	—	3.5
1	1.1	0.051	5.4
2	2.6	0.40	8.4
3	4.6	1.0	13
4	8.0	2.1	23
5	>8.0	3.4	—

TABLE 9221:III. MPN INDEX AND 95% CONFIDENCE LIMITS FOR ALL COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN TEN 10-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 10 (10 mL Each)	MPN Index/ 100 mL	95% Confidence Limits (Exact)	
		Lower	Upper
0	<1.1	—	3.4
1	1.1	0.051	5.9
2	2.2	0.37	8.2
3	3.6	0.91	9.7
4	5.1	1.6	13
5	6.9	2.5	15
6	9.2	3.3	19
7	12	4.8	24
8	16	5.8	34
9	23	8.1	53
10	>23	13	—

Table 9221:IV. MPN Index and 95% Confidence Limits for Various Combinations of Positive Results when Five Tubes Are Used per Dilution (10 mL, 1.0 mL, 0.1 mL)*

Combination of Positives	MPN Index/100 mL	Confidence Limits		Combination of Positives	MPN Index/100 mL	Confidence Limits	
		Low	High			Low	High
0-0-0	<1.8	—	6.8	4-0-3	25	9.8	70
0-0-1	1.8	0.090	6.8	4-1-0	17	6.0	40
0-1-0	1.8	0.090	6.9	4-1-1	21	6.8	42
0-1-1	3.6	0.70	10	4-1-2	26	9.8	70
0-2-0	3.7	0.70	10	4-1-3	31	10	70
0-2-1	5.5	1.8	15	4-2-0	22	6.8	50
0-3-0	5.6	1.8	15	4-2-1	26	9.8	70
1-0-0	2.0	0.10	10	4-2-2	32	10	70
1-0-1	4.0	0.70	10	4-2-3	38	14	100
1-0-2	6.0	1.8	15	4-3-0	27	9.9	70
1-1-0	4.0	0.71	12	4-3-1	33	10	70
1-1-1	6.1	1.8	15	4-3-2	39	14	100
1-1-2	8.1	3.4	22	4-4-0	34	14	100
1-2-0	6.1	1.8	15	4-4-1	40	14	100
1-2-1	8.2	3.4	22	4-4-2	47	15	120
1-3-0	8.3	3.4	22	4-5-0	41	14	100
1-3-1	10	3.5	22	4-5-1	48	15	120
1-4-0	10	3.5	22	5-0-0	23	6.8	70
2-0-0	4.5	0.79	15	5-0-1	31	10	70
2-0-1	6.8	1.8	15	5-0-2	43	14	100
2-0-2	9.1	3.4	22	5-0-3	58	22	150
2-1-0	6.8	1.8	17	5-1-0	33	10	100
2-1-1	9.2	3.4	22	5-1-1	46	14	120
2-1-2	12	4.1	26	5-1-2	63	22	150
2-2-0	9.3	3.4	22	5-1-3	84	34	220
2-2-1	12	4.1	26	5-2-0	49	15	150
2-2-2	14	5.9	36	5-2-1	70	22	170
2-3-0	12	4.1	26	5-2-2	94	34	230
2-3-1	14	5.9	36	5-2-3	120	36	250
2-4-0	15	5.9	36	5-2-4	150	58	400
3-0-0	7.8	2.1	22	5-3-0	79	22	220
3-0-1	11	3.5	23	5-3-1	110	34	250
3-0-2	13	5.6	35	5-3-2	140	52	400
3-1-0	11	3.5	26	5-3-3	170	70	400
3-1-1	14	5.6	36	5-3-4	210	70	400
3-1-2	17	6.0	36	5-4-0	130	36	400
3-2-0	14	5.7	36	5-4-1	170	58	400
3-2-1	17	6.8	40	5-4-2	220	70	440
3-2-2	20	6.8	40	5-4-3	280	100	710
3-3-0	17	6.8	40	5-4-4	350	100	710
3-3-1	21	6.8	40	5-4-5	430	150	1100
3-3-2	24	9.8	70	5-5-0	240	70	710
3-4-0	21	6.8	40	5-5-1	350	100	1100
3-4-1	24	9.8	70	5-5-2	540	150	1700
3-5-0	25	9.8	70	5-5-3	920	220	2600
4-0-0	13	4.1	35	5-5-4	1600	400	4600
4-0-1	17	5.9	36	5-5-5	>1600	700	—
4-0-2	21	6.8	40				

* Results to two significant figures.

where:

V = volume of sample portion at the lowest selected dilution.

When more than three dilutions are used in a decimal series[1] of dilutions, use the following guidelines to select the three most appropriate dilutions and refer to Table 9221:IV. Several illustrative examples (A through G) of combinations of positives are shown in Table 9221:V. First, remove the highest dilution (smallest sample volume) if it has all negative tubes and at least one remaining dilution has a negative tube. Next, remove the lowest dilution (largest sample volume) if it has all positive tubes and at least one remaining dilution has a positive tube. Accord-

TABLE 9221:V. EXAMPLES FOR CHOICE OF THREE COMBINATIONS OF POSITIVES FROM FIVE DILUTIONS

Example	Volume *mL* 10	1	0.1	0.01	0.001	Combination of Positives	MPN Index *No./100 mL*
A	5	**5**	**1**	**0**	0	x-5-1-0-x	330
B	**4**	**5**	**1**	0	0	4-5-1-x-x	48
C	5	2	**5**	**2**	**1**	x-x-5-2-1	7000
D	4	5	**4**	**5**	**1**	x-x-4-5-1	4800
E	5	**4**	**4**	**0**	**1**	x-4-4-1-x	400
F	**4**	**3**	**0**	**1**	**1**	4-3-2-x-x	39
G	4	3	**3**	**2**	**1**	x-x-3-2-1	1700

ing to these guidelines, the three dilutions in Example A are selected by removal of the highest (0.001-mL) and the lowest (10-mL) dilutions.

If the lowest dilution does not have all positive tubes, and several of the highest dilutions have all negative tubes, then remove the highest negative dilutions (Example B).

More than three dilutions may remain after removal of the lowest dilution with all positive tubes and high dilutions with all negative tubes. In this case, if the highest dilution with *all* positive tubes is within two dilutions of the highest dilution with *any* positive tubes, then use the highest dilution with *any* positive tubes and the two immediately lower dilutions. In Example C, the highest dilution with all positive tubes is 0.1 mL, which is within two dilutions of 0.001 mL, which has one positive tube. In Example D, the highest dilution with all positive tubes is 0.01 mL, which is within two decimal dilutions of 0.001 mL, to yield a combination of 4-5-1.

If, after removal of the lowest dilution with all positive tubes, no dilution with all positive reactions remains, then select the lowest two dilutions and assign the sum of any remaining dilutions to the third dilution. In Example E, the highest dilution with all positive tubes contains 10 mL; this dilution was removed in the second step. Four dilutions, none of which have all positive tubes, remain. Under these circumstances, select the two lowest remaining dilutions corresponding to 1 and 0.1 mL sample. For the third dilution, add the number of positive tubes in all higher dilutions (0.01 and 0.001 mL sample), to yield a final combination of 4-4-1.

If no dilution has all positive tubes (Example F), select the lowest two dilutions, corresponding to 10 and 1 mL sample. For the third dilution, add the number of positive tubes in the remaining dilutions (0.1, 0.01, and 0.001 mL sample), to yield a final combination of 4-3-2. If the third dilution is assigned more than five positive tubes, then the selected combination will not be in Table 9221:IV.

If the three dilutions selected are not found in Table 9221:IV, then something in the serial dilution was unusual. In this case, the usual methods for calculating the MPN, presented here, may not apply. If a new sample cannot be collected and an MPN value is still desired, use the highest dilution with at least one positive tube and the two dilutions immediately lower as the three selected dilutions. In Example G, the first selection, 4-3-6 (the outcome from the highest three dilutions), is not in Table 9221:IV because 6 is greater than 5. The second selection, according to the above guidelines, would be 3-2-1. If this second set of selected dilutions is not in Table 9221:IV, then use the following formula to calculate the MPN:

$$-\frac{230.3}{z_s}\log_{10}\left(1-\frac{x_s z_s}{\sum_{j=s}^{K} n_j z_j}\right)$$

where:

j = a dilution,
s = the highest dilution with at least one positive tube,
x_s = the number of positive tubes in the sth dilution,
n_j = the number of tubes in the jth dilution,
z_j = the amount of the original sample inoculated into each tube in the jth dilution,
z_s = the amount of the original sample inoculated into each tube of the sth dilution, and
K = the number of dilutions.

For example, in the series x-x-3-0-0, where the third dilution level (z_s) equals 0.1 mL, $x_s z_s = 0.3$, and $\Sigma\, n_j z_j = 0.555$. Thus, the calculated MPN = 780/100 mL.

This formula also applies to serial dilutions having all positive tubes in a single dilution, and can serve as an approximation for outcomes like 5-5-5-0-0-0, where five tubes are used per dilution, by using just the last four dilutions.

Table 9221:IV shows all but the improbable positive tube combinations for a three-dilution series. In testing 10 samples, there is a 99% chance of finding all the results among these 95 outcomes. If untabulated combinations occur with a frequency greater than 1%, it indicates that the technique is faulty or that the statistical assumptions underlying the MPN estimate are not being fulfilled (e.g., growth inhibition at low dilutions).

The MPN for combinations not appearing in the table, or for other combinations of tubes or dilutions, may be *estimated* as follows: First, select the lowest dilution that does not have all positive results. Second, select the highest dilution with at least one positive result. Finally, select all the dilutions between them. For example, from (10/10, 10/10, 4/10, 1/10, 0/10) use only (–, –, 4/10, 1/10, –), corresponding to 4/10 @ 0.1 mL sample/tube and 1/10 @ 0.01 mL sample/tube. Likewise, from (10/10, 10/10, 10/10, 0/10, 0/10), select only (–, –, 10/10, 0/10, –), corresponding to 10/10 @ 0.1 mL sample/tube and 0/10 @ 0.01 mL

sample/tube. Use only the selected dilutions in the following formula of Thomas:[1]

$$\text{MPN/100 mL (approx.)} = 100 \times P/(N \times T)^{1/2}$$

where:

P = number of positive results,
N = volume of sample in all the negative portions combined, mL, and
T = total volume of sample in the selected dilutions, mL.

That is, $N = \Sigma(n_j - x_j)z_j$, $P = \Sigma x_j$, and $T = \Sigma n_j z_j$, where the summations are over the dilutions selected, and x_j = the number of positive tubes in the jth dilution.

In the first example above,

$$\text{MPN/100 mL (approx.)} = 100 \times 5/(0.69 \times 1.1)^{1/2}$$

$$= 500/0.87 = 570/100 \text{ mL}$$

In the second example above,

$$\text{MPN/100 mL (approx.)} = 100 \times 10/ (0.1 \times 1.1)^{1/2}$$

$$= 1000/0.332 = 3000/100 \text{ mL}$$

The two examples compare well with the true MPNs, 590/100 mL and 2400/100 mL, respectively. The second example is a special case for which an exact solution can be calculated directly for the two selected dilutions.

When it is desired to summarize the results from several samples with a single MPN value, use the geometric mean or the median. The geometric mean is calculated by averaging the logarithmic values; for example, the geometric mean of A, B, and C is 10^L where:

$$L = (\log_{10} A + \log_{10} B + \log_{10} C)/3$$

Mean values are reported as the antilog of L.

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9221 D. Presence–Absence (P–A) Coliform Test

The presence–absence (P–A) test for the coliform group is a simple modification of the multiple-tube procedure. Simplification—by use of one large test portion (100 mL) in a single culture bottle to obtain qualitative information on the presence or absence of coliforms—is justified on the theory that no coliforms should be present in 100 mL of a drinking water sample. Another advantage includes the possibility of examining a larger number of samples per unit of time. Comparative studies with the membrane filter procedure indicate that the P–A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.

The P–A test is intended for use on routine samples collected from distribution systems or water treatment plants.

1. Samples

Collect samples as directed in Sections 9060A and B, using sample containers specified in Section 9030B.19.

2. Presumptive Phase

a. Culture medium:

P–A broth: This medium is commercially available in dehydrated and in sterile concentrated form.

Beef extract	3.0	g
Peptone	5.0	g
Lactose	7.46	g
Tryptose	9.83	g
Dipotassium hydrogen phosphate, K_2HPO_4	1.35	g
Potassium dihydrogen phosphate, KH_2PO_4	1.35	g
Sodium chloride, NaCl	2.46	g
Sodium lauryl sulfate	0.05	g
Bromcresol purple	0.0085	g
Reagent-grade water	1	L

Make this formulation triple strength (3×) when examining 100-mL samples. Dissolve the P–A broth medium in water without heating, using a stirring device. Dispense 50 mL prepared medium into screw-capped 250-mL milk dilution bottles or equivalent containers. A fermentation tube insert is not necessary. Autoclave for 12 min at 121°C, with the total time in the autoclave limited to 30 min or less. Medium pH should be 6.8 ± 0.2 after sterilization. When the P–A medium is sterilized via filtration, a 6× strength medium may be used. Aseptically dispense 20 mL of the 6× medium into a sterile 250-mL dilution bottle or equivalent container.

b. Procedure: Shake sample vigorously for 5 s (approximately 25 times) and inoculate 100 mL into a P–A culture bottle. Mix thoroughly by inverting bottle once or twice to achieve even distribution of the medium throughout the sample. Incubate at 35 ± 0.5°C and inspect after 24 ± 2 and 48 ± 3 h for acid reactions.

c. Interpretation: A distinct yellow color forms in the medium when acidic conditions exist following lactose fermentation. If gas also is being produced, gently shaking the bottle will result in a foaming reaction. Any amount of gas and/or acid constitutes a positive presumptive test requiring confirmation. Simultaneous inoculation of EC broth or EC MUG and then brilliant green lactose bile broth is permitted.

3. Confirmed Phase

The confirmed phase is outlined in Figure 9221:1.

a. Culture medium: Use brilliant green lactose bile (BGLB) fermentation tubes (see 9221B.3*a*).

b. Procedure: Use a sterile wire loop or wooden applicator to transfer all cultures that show acid reaction or acid and gas reaction to BGLB broth for incubation at 35 ± 0.5°C (see 9221B.3).

c. Interpretation: Gas production in BGLB broth culture within 48 ± 3 h confirms the presence of coliform bacteria. Report result as P–A test positive or negative for total coliforms in 100 mL of sample. Drinking water samples that are positive for total coliforms also must be tested for thermotolerant (fecal) coliforms (9221E) or *E. coli* (9221F).

4. Completed Phase

The completed phase, required for nonpotable water sample analysis, is outlined in 9221B.4 and Figure 9221:1.

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9221 E. Fecal Coliform Procedure

Thermotolerant coliforms (those that ferment lactose to produce gas at 44.5°C) were traditionally called *fecal coliforms*, but they also have been documented in organically rich waters or tropical climates in the absence of recent fecal contamination. So, testing for *E. coli*—a specific indicator of fecal contamination—is recommended.

Nevertheless, current regulations may require that thermotolerant (formerly fecal) coliforms be identified and enumerated. In the multiple-tube fermentation technique, this group of organisms is identified by their ability to ferment lactose to produce gas at 44.5°C.

A test for thermotolerant coliforms can be performed using one of the multiple-tube procedures described here or the membrane filter methods described in Sections 9222D and E.

1. Thermotolerant Coliform Test (EC Medium)

The thermotolerant coliform test using EC medium is applicable to investigations of drinking water, stream pollution, raw water sources, wastewater treatment systems, bathing waters, seawaters, and general water-quality monitoring. Do not use EC medium for the direct isolation of thermotolerant coliforms from water. Prior enrichment in a presumptive medium is required for optimum recovery of thermotolerant coliforms. For testing of presumptive coliform colonies growing on solid media, refer to Section 9222G.2.

a. EC medium: Preferably use a dehydrated, commercially available medium.

Tryptose or trypticase	20.0 g

Lactose	5.0 g
Bile salts mixture or bile salts No. 3	1.5 g
Dipotassium hydrogen phosphate, K_2HPO_4	4.0 g
Potassium dihydrogen phosphate, KH_2PO_4	1.5 g
Sodium chloride, NaCl	5.0 g
Reagent-grade water	1 L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. Before sterilization, dispense sufficient medium, in fermentation tubes with an inverted vial, to cover the inverted vial at least one-half to two-thirds after sterilization. Close tubes with metal or heat-resistant plastic caps. Autoclave medium at 121°C for 12 to 15 min. Ensure that inverted vials are free of air bubbles. Medium pH should be 6.9 ± 0.2 after sterilization.

b. Procedure:

1) Gently shake or rotate fermentation tubes or bottles showing gas, growth, or acidity. Using a sterile 3- or 3.5-mm-diam loop or sterile wooden applicator stick, transfer growth from each presumptive or confirmed fermentation tube or bottle to EC broth (see Section 9221B.3).

2) Place all EC tubes in a water bath within 30 min after inoculation. Incubate inoculated EC broth tubes in a water bath at 44.5 ± 0.2°C for 24 ± 2 h. Maintain a sufficient water depth in the water bath incubator to immerse tubes to the upper level of the medium.

c. Interpretation: Gas production and growth in an EC broth culture within 24 ± 2 h or less is considered a positive thermotolerant coliform reaction. Failure to produce gas (with little or no growth) constitutes a negative reaction. If multiple tubes are used, calculate the MPN of thermotolerant coliforms from the number of positive EC broth tubes (as described in 9221C). When using only one tube for subculturing from a single presumptive bottle, report as presence or absence of thermotolerant coliforms. If heavy growth occurs with no gas production, subject the culture to a thermotolerant coliform or *E. coli* test using a different medium.

2. Thermotolerant Coliform Direct Test (A-1 Medium)

a. A-1 medium: This medium may be used for the direct isolation of thermotolerant coliforms from source water, treated wastewater and seawater, but not drinking water. Unlike EC medium, A-1 medium does not require prior enrichment in a presumptive medium for optimum recovery of thermotolerant (fecal) coliforms. Preferably use a dehydrated, commercially available medium.

Lactose	5.0 g
Tryptone	20.0 g
Sodium chloride, NaCl	5.0 g
Salicin	0.5 g
Polyethylene glycol *p*-isooctylphenyl ether*	1.0 mL
Reagent-grade water	1 L

Heat to dissolve solid ingredients, add polyethylene glycol *p*-isooctylphenyl ether, and adjust to pH 6.9 ± 0.1. For 10-mL samples, prepare double-strength medium so the final concentration of ingredients after sample addition is correct. Before sterilization dispense, in fermentation tubes with an inverted vial, sufficient medium to cover the inverted vial at least one-half to two-thirds after sterilization. Close with metal or heat-resistant plastic caps. Sterilize by autoclaving at 121°C for 10 min. Ensure that inverted vials are free of air bubbles. Store in the dark at room temperature for not longer than 7 d. Ignore formation of precipitate during storage.

b. Procedure: Inoculate tubes of A-1 broth as directed in 9221B.2*b*). Incubate for 3 h at 35 ± 0.5°C. Transfer tubes to a water bath at 44.5 ± 0.2°C and incubate for another 21 ± 2 h.

c. Interpretation: Gas production in any A-1 broth culture within 24 h or less is a positive reaction indicating the presence of thermotolerant coliforms. Calculate the MPN of thermotolerant coliforms from the number of positive A-1 broth tubes (as described in 9221C).

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* Triton X-100, Rohm and Haas Co., or equivalent.

9221 F. *Escherichia coli* Procedure Using Fluorogenic Substrate

Escherichia coli is a member of the indigenous fecal flora of warm-blooded animals. The presence of *E. coli* in water is considered a specific indicator of fecal contamination and the possible presence of enteric pathogens. Tests for *E. coli* are applicable to the analysis of drinking water, surface water, groundwater, and wastewater. Testing for *E. coli* can be performed using the multiple-tube procedure described here, by the membrane filter method described in Section 9222G, or by chromogenic enzyme

substrate tests described in Section 9223. Other *E. coli* procedures are presented in 9221G.

For the *E. coli* test using EC-MUG medium, *E. coli* is defined as the species of coliform bacteria possessing the enzyme β-glucuronidase and capable of cleaving the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) with the corresponding release of the fluorogen within 24 ± 2 h or less when grown in EC-MUG medium at 44.5°C.

1. *Escherichia coli* Test (EC-MUG Medium)

Use EC-MUG medium to test for *E. coli* in a total coliform-positive culture. The procedure also can be used to confirm presumptive total coliform colonies growing on solid media, described in Section 9222B.4*f*.

a. EC-MUG medium:

Tryptose or trypticase	20.0	g
Lactose	5.0	g
Bile salts mixture or bile salts No. 3	1.5	g
Dipotassium hydrogen phosphate, K_2HPO_4	4.0	g
Potassium dihydrogen phosphate, KH_2PO_4	1.5	g
Sodium chloride, NaCl	5.0	g
4-Methylumbelliferyl-β-D-glucuronide (MUG)	0.05	g
Reagent-grade water	1	L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. Before sterilization, dispense in tubes that do not fluoresce under long-wavelength (366 nm) ultraviolet (UV) light. An inverted tube is not necessary. Close tubes with metal or heat-resistant plastic caps. Medium pH should be 6.9 ± 0.2 after sterilization for 15 min at 121°C.

b. Procedure:

1) Gently shake or rotate fermentation tubes or bottles showing growth, gas, or acidity. Using a sterile 3- or 3.5-mm-diam metal loop or sterile wooden applicator stick, transfer growth from the fermentation tube or bottle to EC-MUG broth.

2) Place all EC-MUG tubes in water bath within 30 min after inoculation. Incubate inoculated EC-MUG tubes for 24 ± 2 h in a water bath maintained at 44.5 ± 0.2°C. Maintain a sufficient water depth in the water-bath incubator to immerse tubes to the upper level of medium.

c. Interpretation: Examine all tubes exhibiting growth for fluorescence using a 6W, 365 to 366 nm long-wavelength UV lamp. The presence of bright blue fluorescence is considered a positive result for *E. coli*. Growth in the absence of bright blue fluorescence is considered a negative result. To aid in the interpretation of results and to avoid confusion of weak autofluorescence of the medium or glass tubes as a positive response, include in the assay a positive control consisting of a known *E. coli* (MUG-positive) culture, a negative control consisting of a thermotolerant *Klebsiella pneumoniae* (MUG-negative) culture, and an uninoculated medium control. If multiple tubes are used, calculate the MPN for *E. coli* from the number of positive EC-MUG broth tubes as described in Section 9221C. When using only one tube, or subculturing from a single presumptive bottle or colony, report as presence or absence of *E. coli*.

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9221 G. Other *Escherichia coli* Procedures (PROPOSED)

For the *E. coli* test using the GAD reagent, *E. coli* is defined as the species of coliform bacteria possessing the enzyme glutamate decarboxylase (GAD), and capable of producing an alkaline reaction within 4 h in a reagent containing glutamic acid and a lytic agent. The procedure is used to test for *E. coli* after prior enrichment in a medium used for the identification of coliform bacteria. The procedure is particularly useful for determining the presence of MUG-negative strains of *E. coli*, some of which are pathogenic (see also Section 9260F).

1. *Escherichia coli* Test (GAD Procedure)

Use the GAD procedure to test for *E. coli* in a total coliform-positive culture

a. GAD reagent:

L-Glutamic acid	1.0	g
Sodium chloride, NaCl	90.0	g
Bromcresol green	0.05	g
Polyethylene glycol octylphenyl ether*	3.0	mL
Reagent-grade water	1	L

Add ingredients to water and mix thoroughly until all ingredients are dissolved. pH should be 3.4 ± 0.2. The reagent is stable for 2 months when stored at 5°C. It can be filter-sterilized (0.2-μm filter) and treated as a sterile solution.

b. Procedure:

1) Gently shake or rotate presumptive tubes or bottles showing growth, gas, or acidity. Using a graduated pipet, transfer 5 mL broth from the fermentation tube or bottle to 15-mL centrifuge tube.

2) Concentrate the bacterial cells from the broth by centrifugation at 2500 to 3000 × *g* for 10 min. Discard supernatant and resuspend cells in 5 mL phosphate buffer. Reconcentrate cells by centrifugation (2500 to 3000 × *g*, 10 min). Discard supernatant

* Triton X-100, Union Carbide Co., or equivalent.

and add 1.0 mL GAD reagent. Vigorously swirl tube to resuspend cells in GAD reagent.

3) Incubate tubes at 35°C and observe after 1 h. Tubes may be incubated for a maximum of 4 h.

c. Interpretation: Examine all tubes for a distinct color change from yellow to blue. The presence of a blue color is considered a positive result for *E. coli*. To assist in interpretation of results, incorporate in the assay a positive control consisting of a known *E. coli* (GAD-positive) culture, a negative control consisting of a known total coliform organism [e.g., *Enterobacter cloacae* (GAD-negative)] and an uninoculated GAD reagent control. If multiple tubes are used, calculate the MPN for *E. coli* from the number of positive GAD tubes as described in Section 9221C. When using only one tube or a single presumptive bottle, report as presence or absence of *E. coli*.

2. *Escherichia coli* Test (Indole Production)

For the purposes of this test, *E. coli* is defined as the species of coliform bacteria that can produce indole within 24 ± 2 h when grown in tryptone water at 44.5 ± 0.2°C. However, it should be noted that *Klebsiella oxytoca* is indole positive. Use tryptone water and Kovacs' reagent to test for *E. coli* in a total coliform-positive culture.

a. Reagents:

1) *Tryptone water:*

Tryptone	20 g
Sodium chloride, NaCl	5 g
Reagent-grade water	1 L

Add ingredients to water and mix thoroughly until dissolved. Adjust pH to 7.5. Dispense 5-mL portions into tubes, cap, and sterilize for 10 min at 115°C.

2) *Kovacs' reagent:*

p-Dimethylaminobenzaldehyde	5 g
Amyl alcohol (analytical grade)	75 mL
Hydrochloric acid, conc	25 mL

Dissolve aldehyde in alcohol. Cautiously add acid to aldehyde-alcohol mixture and swirl to mix. Store in the dark at 4°C.

CAUTION: **Reagent is corrosive and flammable.** This reagent should be pale yellow to light brown in color. Use of low-quality amyl alcohol may produce a dark-colored reagent; do not use such a reagent.

b. Procedure: Gently shake or rotate presumptive tubes or bottles showing growth, gas, or acidity. Using a sterile 3- or 3.5-mm-diam metal loop or sterile wooden applicator stick, transfer growth from presumptive fermentation tube or bottle to a tube containing 5 mL tryptone water. Incubate inoculated tryptone water tubes in a water bath or incubator maintained at 44.5°C for 24 ± 2 h. After incubation, add 0.2 to 0.3 mL Kovacs' reagent to each tube of tryptone water.

c. Interpretation: Examine all tubes for the appearance of a deep red color in the upper layer. The presence of a red color is considered a positive result for *E. coli*. To assist in interpretation of results, incorporate into the assay a positive control consisting of a known *E. coli* (indole-positive) culture, a negative control consisting of a known total coliform organism [e.g., *Enterobacter cloacae* (indole-negative)] and an uninoculated reagent control. If multiple tubes are used, calculate the MPN for *E. coli* from the number of indole-positive tubes as described in Section 9221C. When using only one tube or a single presumptive bottle, report as presence or absence of *E. coli*.

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9222 MEMBRANE FILTER TECHNIQUE FOR MEMBERS OF THE COLIFORM GROUP*

9222 A. Introduction

The membrane filter (MF) technique is reproducible, can be used to test relatively large sample volumes, and usually yields numerical results more rapidly than the multiple-tube fermentation procedure. The MF technique is useful in monitoring drinking water and a variety of natural waters. However, the MF technique has limitations, particularly when testing waters with high turbidity or large numbers of noncoliform (background) bacteria. If heterotrophic bacteria interference is exhibited, for example, sample results may need to be invalidated and new samples collected. When the MF technique has not been used previously, it is desirable to conduct parallel tests with the method the laboratory is using currently to demonstrate applicability and comparability. There have been many coliform performance studies reported in the literature, and the rates of false positive and negative results can differ among various media. Users should carefully select the medium and procedure that best fits their needs.

1. Definition

As related to the MF technique, the *coliform group* is defined as facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that develop colonies with distinctive characteristics on specific media. Occasionally on m-Endo media, typical sheen colonies may be produced by noncoliform organisms, and atypical colonies (pink, dark red, or nucleated colonies without sheen) may be coliform bacteria; thus, verification of all typical and atypical colonies is recommended. Details of these characteristics are given below for the standard total coliform MF procedure (9222B) and for two procedures for simultaneous detection of total coliforms and *E. coli* (9222H and I).

a. Endo-type agar medium: Coliform bacteria are defined as bacteria that develop red colonies with a metallic (golden-green) sheen within 24 h at 35°C on an Endo-type medium containing lactose. Some members of the total coliform group also produce dark red, mucoid, or nucleated colonies without a metallic sheen. When verified, these are classified as atypical coliform colonies. When purified cultures of coliform bacteria are tested, they produce negative cytochrome oxidase and positive β-galactosidase test reactions.† Generally, pink (non-mucoid), blue, white, or colorless colonies lacking sheen on Endo media are considered noncoliforms by this technique.

b. Dual-chromogen agar medium (mColiBlue24): Coliform bacteria (other than *E. coli*) are defined as those that produce red colonies within 24 h at 35°C on a medium containing lactose and a nonselective dye [2,3,5-triphenoltetrazolium chloride (TTC)]. *E. coli* are distinguished from other coliform bacteria by blue to purple colonies from the action of β-glucuronidase enzyme on 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG), also present in the medium.

c. Fluorogen/chromogen MI medium: Coliform bacteria are defined as bacteria that produce fluorescent colonies upon exposure to longwave ultraviolet (UV) light within 24 h at 35°C on MI medium containing the fluorogen 4-methylumbelliferyl-β-D-galactopyranoside (MUGal). This differential membrane filter medium simultaneously detects and enumerates both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 h based on their specific enzyme activities. Two enzyme substrates—the fluorogen MUGal and a chromogen indoxyl-β-D-glucuronide (IBDG)—are included in the medium to detect the enzymes β-galactosidase and β-glucuronidase produced by TC and *E. coli*, respectively.

2. Applications

The MF technique may be used for testing drinking, surface, ground, swimming pool, and marine waters. Do not use the MF technique to test wastewater receiving only primary treatment unless the sample is diluted, because the high turbidity level may quickly clog the membrane filter before sufficient sample is collected. Chlorinated effluents should have low counts and turbidity. Also, do not use the MF technique to test wastewater containing high levels of toxic metals or toxic organic compounds (e.g., phenols) because such substances may be concentrated by the filter and inhibit coliform growth. For waters other than wastewater, high turbidity levels may clog the filter and high levels of heterotrophic bacteria may interfere with the growth of coliforms on the filter, possibly requiring the use of more than one filter for a sample. This is not a problem with MI medium. To detect stressed total coliforms in treated drinking water and chlorinated secondary or tertiary wastewater effluents, use a method designed for stressed organism recovery (see Section 9212B.1). A modified MF technique for thermotolerant coliforms, some of which are of fecal origin, in chlorinated wastewater (Section 9212) may be used if parallel testing over a 3-month period with the multiple-tube fermentation technique shows comparability for each site-specific type of sample. The standard volume to be filtered for drinking water samples is 100 ± 2.5 mL. This may be distributed among multiple membranes, if necessary. However, for special monitoring purposes (e.g., troubleshooting water quality problems or identifying coliform breakthrough in low concentrations from treatment barriers), it may be desirable to test 1-L samples. If particulates prevent filtering a 1-L sample through a single filter, divide sample into four 250-mL portions for analysis. Total the coliform counts on each membrane to report the number of coliforms per liter. Smaller sample volumes will be necessary for source or recreational waters and wastewater effluents that have much higher coliform densities.

Statistical comparisons of results obtained by the multiple-tube method and the MF technique show that MF is more

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† ONPG is a substrate for the β-galactosidase test.

precise (compare Tables 9221:III and IV with Table 9222:III). Data from each test yield approximately the same water quality information, although numerical results are not identical.

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9222 B. Standard Total Coliform Membrane Filter Procedure

1. Laboratory Apparatus

For MF analyses, use glassware and other apparatus composed of material free from agents that may affect bacterial growth.

a. Sample bottles: See Section 9030B.19.

b. Dilution bottles: See Section 9030B.13.

c. Pipets and graduated cylinders: See Section 9030B.9. Before sterilization, loosely cover opening of graduated cylinders with metal foil or a suitable heavy wrapping-paper substitute. Immediately after sterilization, secure cover to prevent contamination.

d. Containers for culture medium: Use clean borosilicate glass flasks. Any size or shape of flask may be used, but erlenmeyer flasks with metal caps, metal foil covers, or screw caps provide for adequate mixing of the medium contained and are convenient for storage.

e. Culture dishes: Use sterile borosilicate glass or disposable, presterilized plastic petri dishes, 15- × 60-mm, 9- × 50-mm, or other appropriate size. Wrap convenient numbers of clean, glass culture dishes in metal foil if sterilized by dry heat, or suitable heavy wrapping paper when autoclaved. Incubate loose-lidded glass and disposable plastic culture dishes in tightly closed containers to prevent moisture evaporation with resultant drying of medium and to maintain a humid environment for optimum colony development.

Presterilized disposable plastic dishes with tight-fitting lids that meet the specifications above are available commercially and used widely. Reseal opened packages of disposable dish supplies for storage.

f. Filtration units: The filter-holding assembly (constructed of glass, autoclavable plastic, porcelain, stainless steel, or disposable plastic) consists of a seamless funnel fastened to a base by a locking device or by magnetic force. The design should permit the membrane filter to be held securely on the receptacle's porous plate without mechanical damage and allow all fluid to pass through the membrane during filtration. Discard plastic funnels with deep scratches on inner surface or glass funnels with chipped surfaces. Replace damaged screens on stainless steel units.

Wrap the assembly (as a whole or separate parts) in heavy wrapping paper or aluminum foil, or place in commercially available autoclave bags, sterilize via autoclaving, and store until use. Alternatively, expose all surfaces of the previously cleaned and sterilized assembly to UV radiation (2 min exposure) for the initial sanitization before use in the test procedure, or before reusing units in successive filtration series. Field units may be sanitized by dipping in or spraying with alcohol and then igniting or immersing in boiling water for 2 min. Reagent water should be used to avoid hard-water deposits. After submerging unit in boiling water, cool to room temperature before reuse. Do not ignite plastic parts. Sterile, disposable field units also may be used.

For filtration, mount receptacle of filter-holding assembly on a 1-L filtering flask with a side tube or other suitable device (manifold to hold three to six filter assemblies) such that a pressure differential (34 to 51 kPa) can be exerted on the filter membrane. Connect flask to a vacuum line, an electric vacuum pump, a filter pump operating on water pressure, a hand aspirator, or other means of securing a pressure differential (138 to 207 kPa). Connect a flask of approximately the same capacity between filtering flask and vacuum source to trap carry-over water.

g. *Membrane filters:* Use membrane filters with a rated pore diameter to provide complete retention of coliform bacteria (usually 0.45 μm) (for additional specifications, see Section

9020). Only use filter membranes that have been found, through adequate quality control (QC) testing and certification by the manufacturer, to exhibit the following: full retention of the organisms to be cultivated, stability in use, freedom from chemical extractables that may inhibit bacterial growth and development, a satisfactory filtration speed (within 5 min), no significant influence on medium pH (beyond ±0.2 units), and no increase in number of confluent colonies or spreaders compared to control membrane filters. Use membranes grid-marked so bacterial growth is neither inhibited nor stimulated along the grid lines when membranes with entrapped bacteria are incubated on a suitable medium. Preferably use fresh stocks of membrane filters and, if necessary, store them in an environment without temperature and humidity extremes. Obtain no more than a year's supply at any one time.

Preferably use presterilized membrane filters for which the manufacturer has certified that the sterilization technique has neither induced toxicity nor altered the membrane's chemical or physical properties. If membranes are sterilized in the laboratory, autoclave for 10 min at 121°C. At the end of the sterilization period, let the steam escape rapidly to minimize accumulation of water of condensation on filters.

h. Absorbent pads: Disks of filter paper or other material that the manufacturer has certified, by lot, to be of high quality and free of sulfites or other substances that could inhibit bacterial growth. Use pads approximately 48 mm in diameter and of sufficient thickness to absorb 1.8 to 2.2 mL of medium. Some pads may require 3.0 mL of medium. Presterilized absorbent pads or pads subsequently sterilized in the laboratory should release less than 1 mg total acidity (calculated as $CaCO_3$) when titrated to the phenolphthalein endpoint, pH 8.3, using 0.02 *N* NaOH and produce pH levels of 7.0 ± 0.2. Sterilize pads simultaneously with membrane filters available in resealable kraft envelopes, or separately in other suitable containers. Dry pads so they are free of visible moisture before use. See sterilization procedure described for membrane filters above.

i. Forceps: Use smooth blunt forceps, without corrugations on the inner sides of the tips. Sterilize before use by dipping in 95% ethyl or absolute methyl alcohol and flaming.

j. Incubators: Use incubators to provide a temperature of 35 ± 0.5°C and to maintain a humid environment (60% relative humidity).

k. Microscope and light source: To determine colony counts on membrane filters, use a magnification of 10 to 15× and a cool white fluorescent light source adjusted to give maximum sheen discernment. Optimally, use a binocular wide-field dissecting microscope. Do not use a microscope illuminator with optical system for light concentration from an incandescent light source for discerning coliform colonies on Endo-type media.

2. Materials and Culture Media

The need for uniformity dictates the use of commercial dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration. Store opened supplies of dehydrated media in a desiccator (if necessary). Commercially prepared media in liquid form (sterile ampule or other) may be used if known to give equivalent results. See Section 9020 for media QC specifications.

Test each new medium lot against a previously acceptable lot for satisfactory performance (as described in Section 9020B.5*j*). With each new lot of Endo-type medium, verify a minimum 10% of coliform colonies (obtained from natural samples or samples with known additions) to establish the comparative recovery of the medium lot.

Before use, test each batch of laboratory-prepared MF medium for performance with positive and negative culture controls. Check for coliform contamination at the beginning and end of each filtration series by filtering 20 to 30 mL of dilution or rinse water through the filter. If controls indicate contamination, reject all data from affected samples and request new samples.

*a. LES Endo agar:**

Yeast extract	1.2	g
Casitone or trypticase	3.7	g
Thiopeptone or thiotone	3.7	g
Tryptose	7.5	g
Lactose	9.4	g
Dipotassium hydrogen phosphate, K_2HPO_4	3.3	g
Potassium dihydrogen phosphate, KH_2PO_4	1.0	g
Sodium chloride, NaCl	3.7	g
Sodium desoxycholate	0.1	g
Sodium lauryl sulfate	0.05	g
Sodium sulfite, Na_2SO_3	1.6	g
Basic fuchsin	0.8	g
Agar	15.0	g
Reagent-grade water	1	L

CAUTION: Basic fuchsin is a suspected carcinogen and mutagen. Avoid skin contact, ingestion, and exposure to mucous membrane. Follow manufacturer's and Material Safety Data Sheet (MSDS) instructions.

Rehydrate product in 1 L water containing 20 mL 95% ethanol. Do not use denatured ethanol, which reduces background growth and coliform colony size. Bring to a near boil to dissolve agar, then promptly remove from heat and cool to between 45 and 50°C. Do not sterilize by autoclaving. Final pH 7.2 ± 0.2. Dispense in 5- to 7-mL quantities into lower section of 60-mm glass petri dishes or 4- to 6-mL quantities into lower section of 50-mm plastic petri dishes and allow to solidify. If dishes of any other size are used, adjust quantity to give an equivalent depth of 4 to 5 mm. Do not expose poured plates to direct sunlight; refrigerate in the dark, preferably in sealed plastic bags or other containers to reduce moisture loss. Discard unused medium after 2 weeks [or sooner if there is evidence of moisture loss, medium contamination, medium deterioration (darkening of the medium), or surface sheen formation].

b. m-Endo medium:†

Tryptose or polypeptone	10.0	g
Thiopeptone or thiotone	5.0	g
Casitone or trypticase	5.0	g
Yeast extract	1.5	g
Lactose	12.5	g
Sodium chloride, NaCl	5.0	g

* Dehydrated Difco m-Endo Agar LES (No. 273610), or equivalent
† Dehydrated Difco m-Endo Broth MF (No. 274920), dehydrated BBL m-Endo Broth (No. 21119), or equivalent may be used if absorbent pads are used.

Dipotassium hydrogen phosphate, K_2HPO_4	4.375	g
Potassium dihydrogen phosphate, KH_2PO_4	1.375	g
Sodium lauryl sulfate	0.05	g
Sodium desoxycholate	0.10	g
Sodium sulfite, Na_2SO_3	2.10	g
Basic fuchsin	1.05	g
Agar (optional)	15.0	g
Reagent-grade water	1	L

CAUTION: Basic fuchsin is a suspected carcinogen and mutagen. Avoid skin contact, ingestion, or exposure to mucous membranes. Follow manufacturer's and MSDS instructions.

1) Agar preparation—Rehydrate product in 1 L water containing 20 mL 95% ethanol. Heat to near boiling to dissolve agar, promptly remove from heat, and cool to between 45 and 50°C. Dispense 5- to 7-mL quantities into 60-mm sterile glass or 4- to 6-mL quantities into 50-mm plastic petri dishes. If dishes of any other size are used, adjust quantity to give an equivalent depth. Do not sterilize by autoclaving. Final pH should be 7.2 ± 0.2. A precipitate is normal in Endo-type media.

Refrigerate finished medium in the dark, and discard unused agar after 2 weeks.

2) Broth preparation—Prepare as above, omitting agar. Dispense liquid medium (at least 2.0 mL per plate) onto sterile absorbent pads (see absorbent pad specifications, 9222B.1*h*) and carefully remove excess medium by decanting the plate. The broth may have a precipitate but this does not interfere with medium performance if pads are certified free of sulfite or other toxic agents at a concentration that could inhibit bacterial growth. Refrigerated broth in screw-capped bottles or flasks may be stored for up to 4 d.

c. Buffered dilution rinse water: See Section 9050C.1.

3. Samples

Collect samples as directed in Sections 9060A and B.

4. Procedures

a. Selection of sample size: Size of sample will be governed by expected bacterial density and, if applicable, by regulatory requirements.‡ In drinking water analyses, sample size will be limited only by the degree of turbidity or by the noncoliform growth on the medium (Table 9222:I).

An ideal sample volume will yield 20 to 80 total coliform colonies and not more than 200 colonies of all types (typical, atypical, and noncoliform background colonies) on a membrane-filter surface (Table 9222:II). Analyze drinking waters by filtering 100 to 1000 mL, or by filtering replicate smaller sample volumes (e.g., duplicate 50-mL portions or four replicates of 25-mL portions). Analyze other waters by filtering three different volumes (diluted or undiluted), depending on the expected bacterial density. (See Section 9215B.2 for preparation of dilutions.) When less than 10 mL of sample (diluted or undiluted) is to be filtered, add approximately 10 mL sterile buffered dilution water to the funnel and then add sample followed by another 25 to 50 mL dilution water before filtration or pipet the sample volume into sterile dilution water and then filter the entire contents of the dilution bottle. This increase in water volume aids in uniform dispersion of the bacterial suspension over the entire effective filtering surface.

‡ U.S. EPA Total Coliform Rule (June 29, 1989, *Fed. Reg.* 54:27547) prescribes 100-mL for sample size.

TABLE 9222:I. SUGGESTED SAMPLE VOLUMES FOR MEMBRANE FILTER TOTAL COLIFORM TEST

	Volume (X) To Be Filtered *m*L							
Water Source	100	50	10	1	0.1	0.01	0.001	0.0001
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

b. Sterile filtration units: Use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination. A filtration series is interrupted when an interval of 30 min or longer elapses between sample filtrations. After such interruption, treat any further sample filtration as a new filtration series and sterilize all membrane filter holders in use. (See 9222B.1*f* for sterilization procedures and Section 9020B.4*l* and *m* for UV cleaning and safety guidelines.)

c. Filtration of sample: Using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of the base. Carefully place matched funnel unit over base and lock it in place. Filter sample under partial vacuum. With filter still in place, rinse the interior surface of the funnel by filtering three 20- to 30-mL portions of sterile buffered dilution water. Alternatively, rinse funnel by a flow of sterile buffered dilution water from a squeeze bottle (or other appropriate device). This is satisfactory only if the squeeze bottle and its contents do not become contaminated during use. Do not reuse partially filled dilution water bottles. Rinsing between samples prevents carryover contamination. Upon completion of final rinse and filtration process, disengage vacuum, unlock and remove funnel, immediately remove membrane filter with a sterile forceps, and place filter on selected medium with a rolling motion to avoid entrapment of air. Incorrect filter placement is at once obvious, because patches of unstained membrane indicate entrapment of air. Where such patches occur, carefully reseat filter on agar surface.

TABLE 9222:II. NUMBERS OF COLONIES IN THE IDEAL RANGE FOR QUANTITATIVE DETERMINATIONS

	Colony Counting Range	
Test	Minimum	Maximum
Total coliform	20	80
Fecal coliform	20	60
Fecal streptococci	20	100
Enterococci	20	60
E. coli	20	60

Place only one membrane filter per plate. Invert dish, and incubate for 22 to 24 h at 35 ± 0.5°C.

If liquid Endo medium is used, aseptically place a sterile pad in the culture dish and saturate it with at least 2.0 to 3.0 mL of medium (depending on the pad manufacturer), and carefully remove excess medium by decanting the plate. Place prepared filter directly on the pad, invert dish, and incubate Endo plate for 22 to 24 h at 35 ± 0.5°C. If loose-lidded plates are used, place plates in a humid chamber (or humidified incubator).

Differentiation of some colonies may be lost if cultures are incubated beyond 24 h.

Insert a sterile rinse water sample (100 mL) after filtration of a series of 10 samples to check for possible cross-contamination or contaminated rinse water. Incubate the rinse water control membrane culture under the same conditions as the sample.

For nonpotable water samples, preferably rinse and then sanitize the filter unit after each sample (as described above) because of the high number of coliform bacteria present in these samples

d. Alternative enrichment technique: Use this technique with m-Endo media only. Place a sterile absorbent pad in the lid of a sterile culture dish, and pipet at least 2.0 mL lauryl tryptose broth (prepared as directed in Section 9221B.2*a*) to saturate pad. Carefully remove any excess liquid from absorbent pad by decanting plate. Aseptically place filter—through which the sample has been passed—on the pad. Incubate filter, without inverting dish, for 1.5 to 2 h at 35 ± 0.5°C in an atmosphere of at least 60% relative humidity.

Remove enrichment culture from incubator, lift filter from enrichment pad, and roll it onto the m-Endo agar surface, which has been allowed to equilibrate to room temperature. Incorrect filter placement is at once obvious because patches of unstained membrane indicate entrapment of air. Where such patches occur, carefully reseat filter on agar surface. If liquid medium is used, prepare final culture by removing enrichment culture from incubator and separating the dish halves. Place a fresh sterile pad in dish bottom, saturate with at least 2.0 mL of m-Endo medium, and carefully remove excess liquid from absorbent pad by decanting plate. Transfer filter, with same precautions as above, to new pad. Discard used enrichment pad.

With either the agar or the liquid medium, invert dish and incubate for 20 to 22 h at 35 ± 0.5°C. Proceed to ¶ *e* below.

e. Counting: To determine colony counts on m-Endo membrane filters, use a low-power (10 to 15× magnification) binocular wide-field dissecting microscope or other optical device, with a cool white fluorescent light source directed to provide optimal viewing of sheen. The angle of light on the colony affects the detection of sheen for coliform colonies growing on m-Endo plates. Rocking and turning the petri plate reflects light at different angles and aids in detecting sheen on the colony. The typical coliform colony on m-Endo media has a pink to dark-red color with a metallic surface sheen. Count both typical and atypical coliform colonies. The sheen area may vary in size from a small pinhead to complete coverage of the colony surface. Atypical coliform colonies can be dark red, mucoid, or nucleated without sheen. Generally pink, blue, white, or colorless colonies lacking sheen are considered noncoliforms. The total count of colonies (coliform and noncoliform) on Endo-type medium has no consistent relationship to the total number of bacteria present in the original sample. A high count of noncoliform colonies may interfere with the maximum development of coliforms. After 22 h incubation, refrigerating cultures with high densities of noncoliform colonies for 0.5 to 1 h before counting may deter spread of noncoliform growth while aiding sheen discernment. Samples of disinfected water or wastewater effluent may include stressed organisms that grow relatively slowly and produce maximum sheen in 22 to 24 h. Organisms from undisinfected sources may produce sheen at 16 to 18 h.

f. Coliform verification: Occasionally on m-Endo media, typical sheen colonies may be produced by noncoliform organisms, and atypical colonies (pink, dark red, or nucleated colonies without sheen) may be coliforms. Preferably, verify all typical and atypical colony types, but at a minimum, verify at least five typical and five atypical colonies per membrane. For drinking water, verify all colonies on Endo media by swabbing the entire membrane or picking at least five typical colonies and five atypical colonies from a given membrane filter culture. (See Section 9020B.9.) Based on need and sample type, laboratories may incorporate more stringent QC measures (e.g., verify at least one colony from each typical or atypical colony type from a given membrane filter culture, or verify 10% of positive samples). Adjust counts based on verification results. Verification tests are listed below.

1) Lactose fermentation—Transfer growth from each colony, or swab the entire membrane with a sterile cotton swab (for presence–absence results in drinking water samples) and place in lauryl tryptose broth; incubate the lauryl tryptose broth at 35 ± 0.5°C for up to 48 h. Gas formed in lauryl tryptose broth and confirmed in brilliant green lactose broth (see Section 9221B.3*a* for medium preparation) within 48 h verifies the colony as a coliform. Simultaneous inoculation of both media for gas production is acceptable. Inclusion of EC broth inoculation for 44.5 ± 0.2°C incubation will provide information on the presence of thermotolerant coliforms. Use of EC-MUG with incubation at 44.5 ±0.2°C for 24 h will provide information on presence of *E. coli*. (See 9222G for MF partition procedures.)

2) Alternative coliform verifications—Apply this alternative coliform verification procedure to isolated colonies on the Endo membrane filter media. If a mixed culture is suspected or if colony separation is less than 2 mm, streak the growth to m-Endo medium or MacConkey agar to ensure culture purity or submit the mixed growth to the fermentation tube method.

a) Rapid test—A rapid verification of colonies uses test reactions for cytochrome oxidase (CO) and β-galactosidase. Coliform reactions are CO negative and β-galactosidase positive within 4 h incubation of tube culture or micro (spot) test procedure.

b) Commercial multi-test systems—Verify coliform colony by selecting a well-isolated colony, streaking for isolation, and inoculating a pure colony into a multi-test identification system for *Enterobacteriaceae* that includes lactose fermentation and/or β-galactosidase and CO test reactions.

5. Calculation of Coliform Density

Quantitative information only provides a gross estimation of the actual coliform population at collection time due to nonuniform distribution within the matrix. Select the membrane(s) with acceptable number of colonies (Table 9222:II) and not more

than 200 colony-forming units (CFU) of all types per membrane, by the following equation:

$$\text{(Total) coliforms, No./100 mL} = \frac{\text{coliform colonies counted} \times 100}{\text{mL sample filtered}} = \text{No. CFU/100 mL}$$

For drinking water samples, if no total coliform colonies are observed, then report the total coliform colonies counted as "<1 CFU/100 mL."

For nonpotable water samples, if 10.0-, 0.1-, and 0.01-mL portions are examined and all counts are 0, then calculate the number of coliforms per 100 mL that would have been reported

$$1/10 \times 100 = <10 \text{ CFU/100 mL}$$

For verified coliform counts, adjust the initial count based on the positive verification percentage and report as follows:

$$\text{Verified (total) coliforms, No./100 mL} = \frac{\text{number of verified colonies}}{\text{total number of coliform colonies subjected to verification}} \times 100$$

If no colonies are found in a drinking water sample, report "total coliforms absent per 100 mL sample."

a. Water of drinking water quality: While the EPA Total Coliform Rule for drinking water samples requires only a record of total coliform presence or absence in 100-mL samples, quantitative information may sometimes be useful in providing an indication of the magnitude of a contaminating event.

With water of good quality, the occurrence of coliforms generally will be minimal. Therefore, count all coliform colonies (disregarding the lower limit of 20 cited above) and use the formula given above to obtain coliform density.

If confluent growth occurs, covering either the entire filtration area of the membrane or a portion thereof, and colonies are not discrete, report results as "confluent growth with (or without) coliforms." If the total number of bacterial colonies—coliforms plus noncoliforms—exceeds 200 per membrane or if the colonies are not distinct enough for accurate counting, then report results as "too numerous to count" (TNTC) or "confluent," respectively. For drinking water samples using Endo-type media, the presence of coliforms in such cultures may be confirmed (see Section 9224). As an alternative, brush entire filter surface with a sterile loop, applicator stick, or cotton swab and inoculate this growth into a tube of brilliant green lactose bile broth. If gas is produced from the brilliant green bile broth tube within 48 h at 35 ± 0.5°C, coliforms are present. For compliance with the EPA Total Coliform Rule, report confluent growth or TNTC with at least one detectable coliform colony (verification only required with Endo media) as a total coliform positive sample. Report confluent growth or TNTC without detectable coliforms as invalid. For invalid samples, request a new sample from the same location within 24 h and select more appropriate volumes to be filtered per membrane (observing the requirement that the standard drinking water portion is 100 mL) or choose another coliform method that is less subject to heterotrophic bacterial interferences. Thus, to reduce interference from overcrowding, instead of filtering 100 mL per membrane, filter 50-mL portions through two separate membranes, 25-mL portions through four separate membranes, etc. For the Total Coliform Rule, report the sample as total coliform positive if any membrane contains a verified total coliform colony. If a density determination is desired, total the coliform counts observed on all membranes and report as number per 100 mL.

b. Water of other than drinking water quality: As with potable water samples, if no filter has a coliform count falling in the ideal range, total the coliform counts on all filters and report as number per 100 mL. For example, if duplicate 50-mL portions were examined and the two membranes had five and three coliform colonies, respectively, then report the total coliform count as 8 CFU per 100 mL:

$$\frac{[(5 + 3) \times 100]}{(50 + 50)} = 8 \text{ CFU/100 mL}$$

Similarly, if 50-, 25-, and 10-mL portions were examined and the counts were 15, 6, and <1 coliform colonies, respectively, then calculate on the basis of the most nearly acceptable value and report the total coliform count with a qualifying remark as "estimated 30 CFU/100 mL":

$$\frac{[(15) \times 100]}{(50)} = \text{estimated 30 CFU/100 mL}$$

On the other hand, if 10-, 1.0-, and 0.1-mL portions were examined with counts of 40, 9, and <1 coliform colonies, respectively, then select the 10-mL portion only for calculating the coliform density because this filter had a coliform count falling in the acceptable range (see Table 9222:II), and report result as 400 CFU/100 mL:

$$\frac{(40 \times 100)}{10} = 400 \text{ CFU/100 mL}$$

In this last example, if the membrane with 40 coliform colonies also had a total bacterial colony count greater than 200, then report the coliform count as ≥400 CFU/100 mL.

If 10.0-, 1.0-, and 0.1-mL portions were examined with counts of TNTC, 150, and 92 coliform colonies, respectively, then calculate on the basis of the most nearly acceptable value and report, with a qualifying remark, as estimated 92 000 CFU/100 mL:

$$\frac{(92 \times 100)}{0.1} = \text{estimated 92 000 CFU/100 mL}$$

If 1.0-, 0.3-, 0.1-, and 0.03-mL portions were examined with counts of TNTC, TNTC, 78, and 21 coliform colonies, respectively, then sum the total coliform counts on the two filters and divide by the sum of their volume to obtain the final reported value of 76 000 CFU/100 mL:

$$\frac{[(78 + 21) \times 100]}{(0.1 + 0.03)} = 76\,000 \text{ CFU/100 mL}$$

TABLE 9222:III. CONFIDENCE LIMITS FOR MEMBRANE FILTER COLIFORM RESULTS USING 100-mL SAMPLE

Number of Coliform Colonies Counted	95% Confidence Limits	
	Lower	Upper
0	0.0	3.7
1	0.1	5.6
2	0.2	7.2
3	0.6	8.8
4	1.0	10.2
5	1.6	11.7
6	2.2	13.1
7	2.8	14.4
8	3.4	15.8
9	4.0	17.1
10	4.7	18.4
11	5.4	19.7
12	6.2	21.0
13	6.9	22.3
14	7.7	23.5
15	8.4	24.8
16	9.2	26.0
17	9.9	27.2
18	10.7	28.4
19	11.5	29.6
20	12.2	30.8

If 1.0-, 0.3-, and 0.01-mL portions were examined with counts on all portions of TNTC coliform colonies, then calculate using the maximum number of colonies acceptable for quantitative determination for that indicator with the smallest filtration volume and report result as >800 000 CFU/100 mL (for total coliform):

$$\frac{(80 \times 100)}{0.01} = \ > 800\ 000\ \text{CFU/100 mL}$$

c. Statistical reliability of membrane filter results: Although MF results are considered more precise than most probable number (MPN) results, membrane counts may underestimate the number of viable coliform bacteria. Table 9222:III illustrates some 95% confidence limits. These values are based on the assumption that bacteria are distributed randomly and follow a Poisson distribution. For results with counts (c) greater than 20 organisms, calculate the approximate 95% confidence limits using the following normal distribution equations:

$$\text{Upper limit} = c + 2\sqrt{c}$$

$$\text{Lower limit} = c - 2\sqrt{c}$$

6. Bibliography

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9222 C. Delayed-Incubation Total Coliform Procedure

Modification of the standard MF technique permits membrane shipment or transport after filtration to a distant laboratory for transfer to another substrate, incubation, and completion of the test. This delayed-incubation test may be used where it is impractical to apply conventional procedures. It also may be used: (*a*) where it is not possible to maintain the desired sample temperature during transport; (*b*) when the elapsed time between sample collection and analysis would exceed the approved time limit; or (*c*) where the sampling location is remote from laboratory services. (See Section 9060B, Preservation and Storage.)

Independent studies using both fresh- and salt-water samples have shown consistent results between the delayed incubation and standard direct test. Determine the applicability of the delayed-incubation test for a specific water source by comparing with results of conventional MF methods.

To conduct the delayed-incubation test, filter sample in the field immediately after collection, place filter on transport medium, and ship to laboratory. Complete coliform determination in the laboratory by transferring the membrane to standard m-Endo or LES Endo medium, incubating at 35 ± 0.5°C for 20 to 22 h, and counting the typical and atypical coliform colonies that develop. For drinking water samples collected for compliance with the EPA Total Coliform Rule, report the presence or absence of verified coliforms in 100-mL samples. Verify colonies as outlined previously in 9222B.4*f*.

Transport media are designed to keep coliform organisms viable and generally do not permit visible growth during transit time. Bacteriostatic agents in holding/preservative media suppress growth of microorganisms en route but allow normal coliform growth after transfer to a fresh medium.

The delayed-incubation test follows the methods outlined for the total coliform MF procedure, except as indicated below. Two alternative methods are given: one using the m-Endo preservative medium and the other the m-ST holding medium.

1. Apparatus

a. Culture dishes: Use disposable, sterile, plastic petri dishes (9 × 50 mm) with tight-fitting lids. Such containers are lightweight and less likely to break in transit. In an emergency or when plastic dishes are unavailable, use sterile glass petri dishes wrapped in plastic film or similar material. (See 9222B.1*e* for specifications.)

b. Field filtration units: See 9222B.1*f* for specifications. Disinfect by adding methyl alcohol to the filtering chamber, igniting the alcohol, and covering unit to produce formaldehyde. Ultraviolet light disinfection also may be used in the field if an appropriate power source is available (115 V, 60 Hz). Glass or metal filtration units may be sterilized by immersing in boiling water for 2 min. Use reagent water to avoid hard-water deposits. Use a hand aspirator to obtain necessary vacuum.

2. Materials and Transport Media

a. m-Endo methods:

1) *m-Endo preservative medium:* Prepare m-Endo medium as described in 9222B.2*b*. After cooling to below 45°C, aseptically add 3.84 g sodium benzoate (USP grade)/L or 3.2 mL 12% sodium benzoate solution to 100 mL medium. Mix ingredients and dispense in 5- to 7-mL quantities to 9- × 50-mm petri plates. Refrigerate poured plates. Discard unused medium after 96 h.

2) *Sodium benzoate solution*: Dissolve 12 g $NaC_7H_5O_2$ in sufficient reagent water to make 100 mL. Sterilize by autoclaving or by filtering through a 0.22-μm pore size membrane filter. Discard after 6 months.

3) *Cycloheximide:** Optionally, add cycloheximide to m-Endo preservative medium. It may be used for samples that previously have shown overgrowth by fungi, including yeasts. Prepare by aseptically adding 50 mg cycloheximide/100 mL to m-Endo preservative medium. Store cycloheximide solution in refrigerator, and discard after 6 months. **CAUTION: Cycloheximide is a powerful skin irritant. Follow manufacturer's Material Safety Data Sheet (MSDS) instructions for proper handling and storage of this chemical.**

b. m-ST method:

m-ST holding medium:

Sodium phosphate, monobasic, $NaH_2PO_4 \cdot H_2O$	0.1	g
Dipotassium hydrogen phosphate, K_2HPO_4	3.0	g
Sulfanilamide	1.5	g
Ethanol (95%)	10	mL
Tris (hydroxymethyl) aminomethane	3.0	g
Reagent-grade water	1	L

Dissolve ingredients by rehydrating in water. Sterilize by autoclaving at 121°C for 15 min. Final pH should be 8.6 ± 0.2. Dispense at least 2.0 to 3.0 mL (depending on pad manufacturer) to tight-lidded plastic culture dishes containing an absorbent pad, and carefully remove excess liquid from pad by decanting the plate. Store plates in the refrigerator for use within 96 h.

3. Procedure

a. Sample preservation and shipment: Place absorbent pad in bottom of sterile petri dish and saturate with selected coliform holding medium (see 9222C.2). Remove membrane filter from filtration unit with sterile forceps and roll it, grid side up, onto surface of medium-saturated pad. Protect membrane from moisture loss by tightly closing plastic petri dish. Seal loose-fitting dishes with an appropriate sealing tape to prevent membrane dehydration during transit. Place culture dish containing membrane in an appropriate shipping container and send to laboratory for test completion. The sample can be held without visible growth for a maximum of 72 h at ambient temperature on the holding/preservative medium. Visible growth occasionally begins on transport medium when high temperatures are encountered during transit.

b. Transfer and incubation: At the laboratory, transfer filter from holding medium on which it was shipped to a second sterile petri dish containing m-Endo or LES Endo medium and incubate at 35 ± 0.5°C for 20 to 22 h.

* Actidione®, manufactured by the Upjohn Company, Kalamazoo, MI, or equivalent.

4. Estimation of Coliform Density

Proceed as in 9222B.5 above. Record times of collection, filtration, and laboratory examination, and calculate the elapsed time. Report elapsed time with coliform results.

5. Bibliography

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9222 D. Thermotolerant (Fecal) Coliform Membrane Filter Procedure

Thermotolerant coliform (also known as *fecal coliform*) bacterial densities may be determined either by the multiple-tube procedure or by the MF technique. (See Section 9225 for differentiation of *Escherichia coli*, the predominant thermotolerant coliform.) The thermotolerant coliform MF procedure uses an enriched lactose medium and incubation temperature of 44.5 ± 0.2°C for selectivity. Because incubation temperature is critical, submerge waterproofed (plastic bag enclosures) MF cultures in a water bath for incubation at the elevated temperature or use an appropriate solid heat sink incubator or other incubator that is documented to hold the 44.5°C temperature within 0.2°C throughout the chamber over a 24-h period. Areas of application for the fecal coliform method in general are stated in the introduction to the multiple-tube fecal coliform procedures (Section 9221E).

There are limitations to the interpretation of a thermotolerant coliform result from thermal waters (e.g., the tropics), drinking water biofilms, and pulp and paper mill effluent samples where thermotolerant *Klebsiella* have predominated and not been indicative of a sewerage source. As with all coliform results, a sanitary survey should be conducted to identify the most plausible source and public health risk interpretation (see 9222F, *Klebsiella* Membrane Filter Procedure).

According to the World Health Organization, *Klebsiella* spp. do not represent a source of gastrointestinal illness via ingestion of drinking water in the general population. *Klebsiella* spp. detected in drinking water are generally biofilm organisms and are unlikely to represent a health risk.

1. Materials and Culture Medium

a. mFC medium: The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration. Commercially prepared media in liquid form (sterile ampule or other) also may be used if known to give equivalent results. See Section 9020 for QC specifications.

mFC medium:

Tryptose or biosate	10.0 g
Proteose peptone No. 3 or polypeptone	5.0 g
Yeast extract	3.0 g
Sodium chloride, NaCl	5.0 g
Lactose	12.5 g
Bile salts No. 3 or bile salts mixture	1.5 g
Aniline blue	0.1 g
Agar (optional)	15.0 g
Reagent-grade water	1 L

Rehydrate product in 1 L water containing 10 mL 1% rosolic acid in 0.2 *N* NaOH.* Heat to near boiling, promptly remove from heat, and cool to below 50°C. Do not sterilize by autoclaving. If agar is used, dispense 4- to 6-mL quantities to 9- × 50-mm petri plates (approximately 4 to 5 mm deep) and let solidify. Final pH should be 7.4 ± 0.2. Refrigerate finished medium (preferably in sealed plastic bags or other containers to reduce moisture loss) and discard unused broth after 96 h or unused agar after 2 weeks.

Test each medium lot against a previously acceptable lot for satisfactory performance (as described in Section 9020B.5*j*) by making dilutions of a culture of *E. coli* (Section 9020) and filtering appropriate volumes to give 20 to 60 colonies per filter. With each new lot of medium, verify 10 or more colonies obtained from several natural samples to establish the absence of false positives. For most samples, mFC medium may be used without the 1% rosolic acid addition, provided there is no interference with background growth. Such interference may be expected in stormwater samples collected during the first runoff (initial flushing) after a long dry period.

Before use, test each batch of laboratory-prepared MF medium for performance with positive and negative culture controls. Check for coliform contamination at the beginning and end of each filtration series by filtering 20 to 30 mL of dilution or rinse water through filter. If controls indicate contamination, reject all data from affected samples and request new samples.

b. Culture dishes: Tight-fitting plastic dishes are preferred because the membrane filter culture plates are submerged in a water bath during incubation. Place fecal coliform culture plates in plastic bags or seal individual dishes with waterproof (freezer) tape to prevent leakage during submersion. A dry recirculating incubator that can maintain this ±0.2 tolerance can also be used. (Specifications for plastic culture dishes are given in 9222B.1*e*.)

* Rosolic acid reagent will decompose if sterilized by autoclaving. Refrigerate stock solution in the dark and discard after 2 weeks, or sooner, if its color changes from dark red to muddy brown.

TABLE 9222:IV. SUGGESTED SAMPLE VOLUMES FOR MEMBRANE FILTER THERMOTOLERANT COLIFORM OR *E. COLI* TEST

	Volume (X) To Be Filtered *m*L							
Water Source	100	50	10	1	0.1	0.01	0.001	0.0001
Drinking water	X							
Lakes, reservoirs	X	X						
Wells, springs	X	X						
Water supply intake		X	X	X				
Natural bathing waters		X	X	X				
Sewage treatment plant			X	X	X			
Farm ponds, rivers				X	X	X		
Stormwater runoff				X	X	X		
Raw municipal sewage					X	X	X	
Feedlot runoff					X	X	X	
Sewage sludge						X	X	X

c. Incubator: The specificity of the fecal coliform test is related directly to the incubation temperature. Static air incubation may be a problem in some types of incubators because of potential heat layering within the chamber, slower heat transfer from air to the medium, and the slow recovery of temperature each time the incubator is opened during daily operations. To meet the need for greater temperature control, use a water bath, a heat-sink incubator, or a properly designed and constructed incubator shown to give equivalent results. A temperature tolerance of 44.5 ± 0.2°C can be obtained with most types of circulating water baths that are also equipped with a gable top for the reduction of water and heat loss.

2. Procedure

a. Selection of sample size: Select volume of water sample to be examined in accordance with the information in Table 9222:IV. Use sample volumes that will yield counts between 20 and 60 fecal coliform colonies per membrane.

When the bacterial density of the sample is unknown, filter several volumes or dilutions to achieve a countable density. Estimate the volume and/or dilution expected to yield a countable membrane, and select two additional quantities representing one-tenth and ten times (or one-third and three times) this volume, respectively.

b. Filtration of sample: Follow the same procedure and precautions as prescribed under 9222B.4*c*.

c. Preparation of culture dish: Using aseptic technique, place a sterile absorbent pad in each culture dish and pipet at least 2.0 mL mFC medium (prepared as directed above) to saturate pad. Carefully remove any excess liquid from culture dish by decanting the plate. After filtration, aseptically place sample filter on medium-impregnated pad (as described in 9222B).

As a substrate substitution for the nutrient-saturated absorbent pad, add 1.5% agar to mFC broth (as described in 9222B).

d. Incubation: Place prepared dishes in waterproof plastic bags and seal, invert, and submerge petri dishes in water bath; incubate for 24 ± 2 h at 44.5 ± 0.2°C. Anchor dishes below water surface to maintain critical temperature requirements. Place all prepared cultures in the water bath within 30 min after filtration. Alternatively, use an appropriate, accurate solid heat sink or equivalent incubator.

e. Counting: Colonies produced by fecal coliform bacteria on mFC medium are various shades of blue. Nonfecal coliform colonies are gray to cream-colored. Normally, few nonfecal coliform colonies will be observed on mFC medium because of selective action of the elevated temperature and addition of rosolic acid salt reagent. Count colonies with a low-power (10 to 15 magnifications) binocular wide-field dissecting microscope or other optical device.

f. Verification: Verify at a frequency established by the laboratory. Verify typical blue colonies and any atypical grey to green colonies as described in Section 9020 for fecal coliform analysis. Simultaneous inoculation at both temperatures is acceptable.

3. Calculation of Fecal Coliform Density

a. General: Compute the density from the sample quantities that produced MF counts within the desired range of 20 to 60 fecal coliform colonies. This colony density range is more restrictive than the 20 to 80 total coliform range because of larger colony size on mFC medium. Calculate fecal coliform density as directed in 9222B.5. Record fecal coliform densities as CFU per l00 mL.

b. Sediment and biosolids samples: For total solids (dry weight basis), see Section 2540G.

Calculate fecal coliforms per gram dry weight for biosolids analysis as follows:

$$\text{Fecal coliform, CFU /g dry weight} = \frac{\text{colonies counted}}{(\text{dilution chosen}) \times (\%\ \text{dry solids})}$$

where dilution and % dry solids are expressed in decimal form.

Example 1: There were 22 colonies observed on the 1:10 000 dilution plate of a biosolids with 4% dry solids.

$$\frac{22}{(0.0001)\ (0.04)} = 5.5 \times 10^6\ \text{CFU / g dry weight}$$

If no filter has a coliform count falling in the ideal range (20 to 60), total the coliform counts on all countable filters, and report as fecal coliforms per gram dry weight:

Example 2: There were 18 colonies observed on the 1:10 000 dilution plate and 2 colonies observed on the 1:100 000 dilution plate of a biosolids sample with 4% dry solids.

$$\frac{(18 + 2)}{(0.0001 + 0.00001)\ (0.04)} = 4.5 \times 10^6$$

To compute a geometric mean of samples, convert coliform densities of each sample to $\log_{10}$ values. Determine the geometric mean for the given number of samples† by averaging the

† Usually seven if collecting for the EPA Pathogen Reduction Rule, 40 CFR Part 503.

$\log_{10}$ values of the coliform densities and taking the antilog of that value.

4. Bibliography

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9222 E. Delayed-Incubation Thermotolerant (Fecal) Coliform Procedure

This delayed-incubation procedure is similar to the delayed-incubation total coliform procedure (9222C). Use the delayed-incubation test only when the standard immediate thermotolerant coliform test cannot be performed (i.e., where the appropriate field incubator is unavailable, or where, under certain circumstances, a specialized laboratory service is advisable to examine, confirm, or speciate the suspect colonies).

Results obtained by this delayed method have been consistent with results from the standard fecal coliform MF test under various laboratory and field use conditions. However, determine test applicability for a specific water source by comparison with the standard MF test, especially for saline waters, chlorinated wastewaters, and waters containing toxic substances.

To conduct the delayed-incubation test, filter sample in the field immediately after collection, place filter on m-ST holding medium (see 9222C.2*b*), and ship to the laboratory. Complete thermotolerant coliform test by transferring filter to mFC medium, incubating at 44.5°C for 24 ± 2 h, and counting thermotolerant coliform colonies.

The m-ST medium keeps thermotolerant coliform organisms viable but prevents visible growth during transit. Membrane filters can be held for up to 3 d on m-ST holding medium with little effect on the thermotolerant coliform counts.

1. Apparatus

a. Culture dishes: See 9222C.1*a* for specifications.
b. Field filtration units: See 9222C.1*b*.

2. Materials and Transport Medium

a. m-ST medium: Prepare as described in 9222C.2*b*.
b. mFC medium: Prepare as described in 9222D.1*a*.

3. Procedure

a. Membrane filter transport: Using aseptic technique, place an absorbent pad in a tight-lid plastic petri dish and saturate with m-ST holding medium. After filtering sample, remove the membrane filter from filtration unit and place it on medium-saturated pad. Use only tight-lid dishes to prevent moisture loss; however, avoid having excess liquid in the dish. Place culture dish containing the filter in an appropriate shipping container and send to laboratory. Membranes can be held on the transport medium at ambient temperature for a maximum of 72 h with little effect on fecal coliform counts.

b. Transfer: At the laboratory, remove membrane from holding medium and place it in another dish containing mFC medium.

c. Incubation: After transfering filter to mFC medium, place tight-lid dishes in waterproof plastic bags, invert, and submerge in a water bath at 44.5 ± 0.2°C for 24 ± 2 h, or use a solid heat sink or equivalent incubator.

d. Counting: Colonies produced by fecal coliform bacteria are various shades of blue. Nonfecal coliform colonies are gray to cream-colored. Count colonies with a binocular wide-field dissecting microscope at 10 to 15× magnification.

e. Verification: Verify colonies at a frequency established by the laboratory. Verify typical blue colonies and any atypical (grey to green) colonies as described in Section 9020 for fecal coliform analysis.

4. Estimation of Fecal Coliform Density

Count as directed in 9222D.2*e* above and compute fecal coliform density as described in 9222D.3. Record time of collection, filtration, and laboratory examination, and calculate and report elapsed time.

5. Bibliography

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9222 F. *Klebsiella* Membrane Filter Procedure

Klebsiella bacteria belong to the family *Enterobacteriaceae* and are included in the total coliform group. The outermost layer of *Klebsiella* bacteria consists of a large polysaccharide capsule (a characteristic that distinguishes this genus from most other bacteria in this family); this capsule provides some measure of protection from disinfectants. *Klebsiella* bacteria are commonly associated with coliform regrowth in large water supply distribution systems.

Klebsiella bacteria may be opportunistic pathogens that can give rise to bacteremia, pneumonia, urinary tract, and several other types of human infection where spread is associated with frequent handling of hospital patients—especially those with impaired immune systems, patients with burns or excessive wounds, etc. *Klebsiella* spp. are also excreted in the feces of many healthy humans and animals, and they are readily detected in sewage-polluted waters. Approximately 60 to 80% of all *Klebsiella* from feces and from clinical specimens are positive in the fecal coliform test and are *Klebsiella pneumoniae*.

Klebsiella bacteria also are widely distributed in nature, occurring in soil, water, grain, vegetation, etc. Wood pulp, paper mills, textile finishing plants, and sugar-cane processing operations contain large numbers of *Klebsiella* sp. in their effluents (10^4 to 10^6), and *Klebsiella* sp. are often the predominant coliform in such effluents.

Rapid quantitation may be achieved in the MF procedure by modifying mFC agar base through substitution of inositol for lactose and adding carbenicillin or by using mKleb agar. These methods reduce the necessity for biochemical testing of pure strains. Preliminary verification of differentiated colonies is recommended.

1. Apparatus

a. Culture dishes: See 9222B.1*e* for specifications.
b. Filtration units: See 9222B.1*f*.

2. Materials and Culture Medium

a. Modified mFC agar (mFCIC agar): This medium may not be available in dehydrated form and may require preparation from the basic ingredients:

Tryptose or biosate	10.0 g
Proteose peptone No. 3 or polypeptone	5.0 g
Yeast extract	3.0 g
Sodium chloride, NaCl	5.0 g
Inositol	10.0 g
Bile salts No. 3 or bile salts mixture	1.5 g
Aniline blue	0.1 g
Agar	15.0 g
Reagent-grade water	1 L

Heat medium to boiling, and add 10 mL 1% rosolic acid dissolved in 0.2 *N* NaOH. Cool to below 45°C, and add 50 mg carbenicillin.* Dispense aseptically in 4- to 6-mL quantities into 9- × 50-mm plastic petri dishes (approximate depth of 4 to 5 mm). Refrigerate until needed. Discard unused agar medium after 2 weeks. Do not sterilize by autoclaving. Final pH should be 7.4 ± 0.2.

b. mKleb agar:

Phenol red agar	31.0 g
Adonitol	5.0 g
Aniline blue	0.1 g
Sodium lauryl sulfate	0.1 g
Reagent-grade water	1 L

Sterilize by autoclaving for 15 min at 121°C. After autoclaving, cool to 50°C in a water bath; add 20 mL 95% ethyl alcohol (not denatured) and 0.05 g filter-sterilized carbenicillin/L. Shake thoroughly and dispense aseptically into 9- × 50-mm plastic culture plates. The final pH should be 7.4 ± 0.2. Refrigerated medium can be held for 20 d at 4 to 8°C.

3. Procedure

a. See 9222B.4 for selection of sample size and filtration procedure. Select sample volumes that will yield counts between 20 and 60 *Klebsiella* colonies per membrane. Place membrane filter on agar surface; incubate for 24 ± 2 h at 35 ± 0.5°C. *Klebsiella* colonies on mFCIC agar are blue or bluish-gray. Most atypical colonies are brown or brownish. Occasional false-positive occurrences are caused by *Enterobacter* species. *Klebsiella* colonies on mKleb agar are deep blue to blue gray, whereas other colonies most often are pink or occasionally pale yellow. Count colonies with a low-power (10 to 15× magnification) binocular wide-field dissecting microscope or other optical device.

b. Verification: Verify *Klebsiella* colonies from the first set of samples from ambient waters and effluents, and when *Klebsiella* is suspect in water supply distribution systems. Verify a minimum of five typical colonies by transferring growth from a colony or pure culture to a commercial multi-test system for gram-negative speciation. Key tests for *Klebsiella* are citrate (positive), motility (negative), lysine decarboxylase (positive), ornithine decarboxylase (negative), and urease (positive). A *Klebsiella* strain that is indole-positive, liquefies pectin, and demonstrates a negative fecal coliform response is most likely of nonfecal origin.

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9222 G. MF Partition Procedures

1. *Escherichia coli* Partition Methods

a. Applications: Escherichia coli is a member of the thermotolerant coliform group of bacteria; its presence is indicative of fecal contamination. Rapid quantitation and verification for *E. coli* may be achieved for a total-coliform- or fecal-coliform-positive MF sample by using media containing 4-methylumbelliferyl-β-D-glucuronide (MUG). In this method, *E. coli* is defined as any coliform that produces the enzyme β-glucuronidase and hydrolyzes the MUG substrate to produce a blue fluorescence.

When examining drinking water samples, use one of the partition methods to determine the presence of *E. coli* from a total-coliform-positive MF sample on Endo-type media. When examining wastewater and other nonpotable water samples, use one of the partition methods to determine the presence of *E. coli* from thermotolerant (fecal)-coliform-positive MF samples on mFC media.

b. Apparatus:

1) *Culture dishes:* See 9222B.1*e*.
2) *Filtration units:* See 9222B.1*f*.
3) *Forceps:* See 9222B.1*i*.
4) *Incubator:* See 9222B.1*j*.
5) *Ultraviolet lamp,* long wave (366 nm), 6 W.
6) *Microscope* and *light source:* See 9222B.1*k*.

c. Materials and culture medium:

1) *Nutrient agar with MUG (NA-MUG):*

Peptone	5.0	g
Beef extract	3.0	g
Agar	15.0	g
4-methylumbelliferyl-β-D-glucuronide	0.1	g
Reagent-grade water	1	L

Add dehydrated ingredients to reagent-grade water, mix thoroughly, and heat to dissolve. Sterilize by autoclaving for 15 min at 121°C. Dispense 4- to 6-mL quantities aseptically into 50-mm plastic culture plates (approximate depth of 4 to 5 mm) and allow to solidify. Final pH should be 6.8 ± 0.2. Refrigerated prepared medium may be held for 2 weeks.

2) *EC broth with MUG (EC-MUG):*

Tryptose or trypticase	20.0	g
Lactose	5.0	g
Bile salts mixture or bile salts No. 3	1.5	g
Dipotassium hydrogen phosphate, K_2HPO_4	4.0	g
Potassium dihydrogen phosphate, KH_2PO_4	1.5	g
Sodium chloride, NaCl	5.0	g
4-methylumbelliferyl-β-D-glucuronide	0.05	g
Reagent-grade water	1	L

Add dehydrated ingredients to reagent-grade water, mix thoroughly, and heat to dissolve. pH should be 6.9 ± 0.2 after sterilization. Before sterilization, dispense into culture tubes, and cap with metal or heat-resistant plastic caps.

d. Procedure:

1) Selection of sample size and filtration procedure—See 9222B.4.

2) Total coliform verification—For drinking water samples using Endo-type medium, total coliform verification procedures can be performed before or after the partition method. Swab surface growth on the m-Endo filter or, if quantification is desired, transfer small portions of each target colony on m-Endo filter to the appropriate total coliform verification medium using a sterile needle. Alternatively, after transferring filter to NA-MUG media, incubating, and reading the results on this media, either transfer individual colonies, swab surface growth on filter, or place whole filter into appropriate total coliform verification medium. (See 9222B.4 for total coliform verification procedures.)

3) Partition method for *E. coli* determination—If NA-MUG medium is used, aseptically transfer membrane filter with at least one coliform-positive colony to NA-MUG plate. If quantification is desired, mark each sheen colony with a fine-tipped marker on reverse side of plate or puncture a hole in membrane filter adjacent to the colony with a sterile needle after transfer of membrane to NA-MUG medium. Incubate NA-MUG at 35 ± 0.5°C for 4 h. If EC-MUG medium is used, use aseptic technique to transfer total coliform-positive colonies on the membrane filter to a tube containing EC-MUG medium by one of the following methods: (*a*) remove membrane containing total coliform colonies from the substrate with sterile forceps and carefully curl and insert membrane into tube of EC-MUG medium, (*b*) swab entire membrane filter surface with a sterile cotton swab and transfer the inoculum to EC-MUG medium (do not leave cotton swab in EC-MUG medium), or (*c*) if quantification is desired, inoculate individual total coliform-positive colonies into separate EC-MUG tubes. Incubate EC-MUG media at 44.5 ± 0.2°C for 24 ± 2 h.

Observe individual colonies or tubes using a long-wavelength (366-nm) UV light source, preferably containing a 6-W bulb. The presence of a bright blue fluorescence in the tube, on the

periphery (outer edge) of a colony, or observed from the back of the plate is a positive response for *E. coli*. Record presence or absence of fluorescence, or if quantification is desired, count and record the number of target colonies. For nonpotable water samples, this partition method can be used to determine *E. coli* from the fecal coliform MF procedure using mFC medium for initial isolation before transfer to NA-MUG or EC-MUG medium. The procedure is the same as the above, with the exception of the total coliform verification process.

For the EC-MUG method, a positive control consisting of a known *E. coli* (MUG-positive) culture, a negative control consisting of a thermotolerant *Klebsiella pneumoniae* (MUG-negative) culture, and an uninoculated medium control may be necessary to interpret sample results and to avoid confusion of weak autofluorescence of the medium as a positive response. (See Section 9221F.)

2. Thermotolerant (Fecal) Coliform Partition Method

a. Applications: In a drinking water sample, thermotolerant coliform determination can be performed from a total-coliform-positive MF filter within 24 h. This technique may be applicable to other waters if warranted.

b. Materials and culture medium: EC broth. See Section 9221E.1*a.*

c. Procedure:

1) Selection of sample size and filtration procedure—See 9222B.4.

2) Total coliform verification—Verify total coliforms before using the fecal coliform partition method. Swab surface growth on the total-coliform-positive filter or, if quantification is desired, transfer small portions of each target colony on the filter to the appropriate total coliform verification medium using a sterile needle. See 9222B.4*f* for total coliform verification procedures.

3) Partition method for fecal coliform determination—Using aseptic technique, transfer total-coliform-positive colonies from the membrane filter to a tube containing EC medium by one of the following methods: (*a*) remove membrane containing the total coliform colonies from the substrate with sterile forceps and carefully curl and insert membrane into tube of EC medium, (*b*) swab the entire membrane filter surface with a sterile cotton swab and transfer the inoculum to EC medium (do not leave the cotton swab in the medium), or (*c*) if quantification is desired, inoculate individual total coliform-positive colonies into separate EC tubes. Incubate inoculated EC broth in a water bath at 44.5 ± 0.2°C for 24 ± 2 h. Place all EC tubes in water bath within 30 min after inoculation. Maintain a sufficient water depth in water bath incubator to immerse tubes to upper level of the medium. Gas production in an EC broth culture in 24 h or less is considered a positive response for fecal coliform bacteria.

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9222 H. Simultaneous Detection of Total Coliform and *E. coli* by Dual–Chromogen Membrane Filter Procedure (PROPOSED)

1. Laboratory Apparatus

For MF analyses, use glassware and other apparatus composed of material free from agents that may affect bacterial growth.

a. Sample bottles: See Section 9030B.19.

b. Dilution bottles: See Section 9030B.13.

c. Pipets, sample containers, and graduated cylinders: See 9222B.1*c*.

d. Culture dishes: See 9222B.1*e*.

e. Filtration units: See 9222B.1*f*.

f. Membrane filters: See 9222B.1*g*.

g. Absorbent pads: See 9222B.1*h*.

h. Forceps: See 9222B.1*i*.

i. Incubators: See 9222B.1*j*.

2. Culture Medium

Purchase this medium* from a commercial vendor; it cannot be prepared from basic ingredients. See Section 9020 for media QC specifications.

Before use, test each lot with positive and negative culture controls. Check for coliform contamination at the beginning and end of each filtration series by filtering 20 to 30 mL of dilution or rinse water through the filter. If controls indicate contamination, reject all data from affected samples and request new samples.

* m-ColiBlue24® broth, Hach Company, Loveland, CO; Millipore Corporation, Billerica, MA.

L-Methionine	0.1	g
Methylene blue	0.016	g
Casitone	8.0	g
Yeast extract	0.5	g
Lactose	0.6	g
Sodium chloride, NaCl	3.0	g
Dipotassium hydrogen phosphate, K_2HPO_4	1.75	g
Potassium dihydrogen phosphate, KH_2PO_4	1.25	g
Triphenyl tetrazolium chloride	0.07	g
Sodium pyruvate	1.0	g
Erythromycin	3.0	g
Octyphenol ethoxylate†	0.5	g
Magnesium sulfate, $MgSO_4$	0.3	g
5-bromo-4-chloro-3-indolyl-β-D–glucuronic acid (proprietary)		
Sodium azide	0.02	g
Cyclohexylammonium salt	0.2	g
Reagent-grade water	1	L

CAUTION: Sodium azide is highly toxic and mutagenic. Follow manufacturer's MSDS instructions for proper storage and handling of this medium.

Mix broth gently by inverting ampules two or three times before dispensing. Pour liquid medium (approximately 2.0 mL per plate) evenly onto sterile absorbent pads and place lid on petri dish. Final pH should be 7.0 ± 0.2.

3. Procedure

a. Selection of sample size: See 9222B.4*a*.

b. Sterile filtration units: See 9222B.4*b*.

c. Filtration of sample: See 9222B.4*c*.

d. Counting: To count colonies on membrane filters, use a low-powered (10 to 15×) binocular wide-field dissecting microscope or other optical device with a cool white fluorescent light source directed to provide optimal viewing. Count all red and blue to purple colonies under normal/ambient light and record as the total coliform result. Count only blue to purple colonies and record as *E. coli* result. Clear or white colonies are considered noncoliform colonies. A high noncoliform count may interfere with the development of coliform colonies.

† Triton X-114.

e. Coliform verification: For drinking water, total coliform and colony verification is not required for this medium. For waters other than drinking water, verify at a frequency established by the laboratory (see Section 9020B.9). Based on need and sample type, laboratories may incorporate more stringent QC measures (e.g., verify at least one colony from each typical or atypical colony type from a given membrane filter culture, verify 10% of the positive samples) (see Section 9020B.9). Adjust counts based on verification results. Verification tests are listed in 9222B.4*f*.

4. Calculation of Coliform Density

See 9222B.5. Calculate the final values using the formula:

$$E.coli/100\text{ mL} = \frac{\text{number of blue-purple colonies}}{\text{volume of sample filtered (mL)}} \times 100$$

$$\text{Total coliforms/100 mL} = \frac{\text{number of red } \textit{and} \text{ blue to purple colonies}}{\text{volume of sample filtered (mL)}} \times 100$$

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9222 I. Simultaneous Detection of Total Coliform and *E. coli* by Fluorogen/Chromogen Membrane Filter Procedure (PROPOSED)

1. Laboratory Apparatus

For MF analyses, use glassware and other apparatus composed of material free from agents that may affect bacterial growth.

a. Sample bottles: See Section 9030B.19.

b. Dilution bottles: See Section 9030B.13.

c. Pipets, sample containers, and graduated cylinders: See 9222B.1*c*.

d. Culture dishes: See 9222B.1*e*.

e. Filtration units: See 9222B.1*f*.

f. Membrane filters: See 9222B.1*g*.

g. Absorbent pads: See 9222B.1*h*.

h. Forceps: See 9222B.1*i*.
i. Incubators: See 9222B.1*j*.

2. Culture Media

The need for uniformity dictates the use of commercial dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration. Store opened supplies of dehydrated media in a desiccator (if necessary). Commercially prepared media in liquid form (sterile ampule or other) may be used if known to give equivalent results. See Section 9020 for media QC specifications.

Test each new medium lot against a previously acceptable lot for satisfactory performance (as described in Section 9020B).

a. Cefsulodin solution, 1 mg/1 mL: Add 0.02 g of cefsulodin to 20 mL reagent-grade distilled water, sterilize using a 0.22-μm syringe filter, and store in a sterile tube at 4°C until needed. Prepare fresh solution each time MI medium is made. Do not save the unused portion.

*b. MI agar:**

Proteose peptone No. 3	5.0	g
Yeast extract	3.0	g
β-D-Lactose	1.0	g
4-Methylumbelliferyl-β-D-galactopyranoside (MUGal) (final concentration 100 μg/mL)	0.1	g
Indoxyl-β-D-glucuronide (IBDG) (final concentration 320 μg/mL)	0.32	g
NaCl	7.5	g
K_2HPO_4	3.3	g
KH_2PO_4	1.0	g
Sodium lauryl sulfate	0.2	g
Sodium desoxycholate	0.1	g
Agar	15.0	g
Reagent-grade distilled water	1	L

Autoclave medium for 15 min at 121°C, and add 5 mL of freshly-prepared cefsulodin (¶ *a* above) solution (5 μg/mL final concentration) per liter of tempered agar medium. Final pH should be 6.95 ± 0.2. Pipet medium into 9- × 50-mm petri dishes (5 mL/plate). Store plates at 4°C for up to 2 weeks.

c. MI broth:† Use same ingredients as MI agar, but omit agar. Prepare and sterilize, and add cefsulodin by the methods described for MI agar. Alternatively, the broth can be filter-sterilized. Final pH should be 7.05 ± 0.2. Place absorbent pads in 9- × 50-mm petri dishes and saturate with 2.0 to 3.0 mL MI broth containing 5 μg/mL final concentration of cefsulodin. Store plates in the refrigerator and discard after 96 h. Pour off excess broth before using the plates.

3. Procedure

a. Selection of sample size: See 9222B.4*a*.
b. Sterile filtration units: See 9222B.4*b*.
c. Filtration of sample: See 9222B.4*c*, with the following exception: incubate MI broth plates *grid-side up* at 35 ± 0.5°C for 22 to 24 h.

* BBL™ MI prepared plates (No. 214986), or equivalent.
† Dehydrated Difco™ MI Broth (No. 214882), or equivalent.

d. Counting: To count colonies on membrane filters, use a low-powered (10 to 15×) binocular wide-field dissecting microscope or other optical device with a cool white fluorescent light source directed to provide optimal viewing. Count all blue colonies on each MI plate under normal/ambient light and record as *E. coli* results. Positive results that occur in less than 24 h are valid, but results cannot be recorded as negative until the 24-h incubation period is complete. Expose each MI plate to long-wave UV light (366 nm), and count all fluorescent colonies [blue/green fluorescent *E. coli*, blue/white fluorescent TC other than *E. coli*, and blue/green with fluorescent edges (also *E. coli*)] to obtain the TC count. Record the data. Add any blue, nonfluorescent colonies (if any) found on the same plate to the TC count.

Calculate the final values using the formula:

$$E.coli/100\text{ mL} = \frac{\text{number of blue colonies}}{\text{volume of sample filtered (mL)}} \times 100$$

$$\text{TC}/100\text{ mL} = \frac{\text{number of fluorescent colonies} + \text{number of blue, non-fluorescent colonies (if any)}}{\text{volume of sample filtered (mL)}} \times 100$$

e. Coliform verification: For drinking water, total coliform colony verification is not required for this medium on coliform colonies from MI media. For waters other than drinking water, verify at a frequency established by the laboratory (see Section 9020B.9). Based on need and sample type, laboratories may incorporate more stringent QC measures (e.g., verify at least one colony from each typical or atypical colony type from a given membrane filter culture, verify 10% of positive samples) (see Section 9020B.9). Adjust counts based on verification results. Verification tests are listed in 9222B.4*f*.

4. Calculation of Coliform Density

See 9222B.5.

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9223 ENZYME SUBSTRATE COLIFORM TEST*

9223 A. Introduction

The enzyme substrate test utilizes hydrolyzable substrates for the simultaneous detection of total coliform bacteria and *Escherichia coli* enzymes. When the enzyme technique is used, the total coliform group is defined as all bacteria possessing the enzyme β-D-galactosidase, which cleaves the chromogenic substrate, resulting in release of the chromogen. *Escherichia coli* are defined as bacteria giving a positive total coliform response and possessing the enzyme β-glucuronidase, which cleaves a fluorogenic substrate, resulting in the release of the fluorogen. The test can be used in a multiple-tube, multi-well, or a presence-absence (single 100-mL sample) format.

1. Principle

a. Total coliform bacteria: Chromogenic substrates, such as ortho-nitrophenyl-β-D-galactopyranoside (ONPG) or chlorophenol red-β-D-galactopyranoside (CPRG), are used to detect the enzyme β-D-galactosidase, which is produced by total coliform bacteria. The β-D-galactosidase enzyme hydrolyzes the substrate and produces a color change, which indicates a positive test for total coliforms at 18 and 24 h (ONPG) or 24 h (CPRG) without additional procedures. Noncoliform bacteria, such as *Aeromonas, Flavobacterium,* and *Pseudomonas* species, may produce small amounts of the enzyme β-D-galactosidase, but are suppressed and generally will not produce a positive response within the incubation time unless more than 10^4 colony-forming units (CFU)/mL (10^6 CFU/100 mL) are present.

b. Escherichia coli: A fluorogenic substrate, such as 4-methylumbelliferyl-β-D-glucuronide (MUG), is used to detect the enzyme β-glucuronidase, which is produced by *E. coli.* The β-glucuronidase enzyme hydrolyzes the substrate and produces a fluorescent product when viewed under long-wavelength (365-nm) ultraviolet (UV) light. The presence of fluorescence indicates a positive test for *E. coli.* Some strains of *Shigella* and *Salmonella* spp. also may produce a positive fluorescence response. Because *Shigella* and *Salmonella* spp. are overt human pathogens, this is not considered a detriment for testing the sanitary quality of water.

2. Applications

The enzyme substrate coliform test is recommended for the analysis of drinking and source water samples. Formulations also are available for the analysis of marine waters. Initially, laboratories planning to use this procedure should conduct parallel quantitative testing (including seasonal variations) with one of the standard coliform tests to assess the effectiveness of the test for the specific water type being analyzed and to determine the comparability of the two techniques. This is particularly important when testing source waters.

Water samples containing humic or other material may be colored. If there is background color, compare inoculated tubes to a control tube containing only water sample. In certain waters, high calcium salt content can cause precipitation but this should not affect the reaction.

Do not use the enzyme substrate test to verify presumptive coliform cultures or membrane filter colonies, because the substrate may be overloaded by the heavy inoculum of weak β-D-galactosidase-producing noncoliforms, causing false-positive results.

* Approved by Standard Methods Committee, 2004.
Joint Task Group: Carol J. Palmer (chair), Terry C. Covert, Robert E. Grant, Nancy H. Hall, Eugene W. Rice, Bruce M. Roll, Helena M. Solo-Gabriele.

9223 B. Enzyme Substrate Test

1. Substrate Media

Formulations are available commercially* in premeasured packets for presence-absence or quantification† and disposable tubes for the multiple-tube procedure.* The need for good quality assurance and uniformity requires the use of a commercial substrate medium. Avoid prolonged exposure of the substrate to direct sunlight. Store media according to directions and use before expiration date. Discard colored media.

2. Procedure

a. Multiple-tube procedure: Select the appropriate number of tubes per sample with predispensed media for the multiple-tube test and label. Follow manufacturer's instructions for preparing serial dilutions for various formulations. Aseptically add 10 mL sample to each tube, cap tightly, and mix vigorously to dissolve. The mixture remains colorless with ONPG-based tests and turns yellow with the CPRG format. Some particles may remain undissolved throughout the test; this will not affect test performance. Incubate at 35 ± 0.5°C for period specified by substrate manufacturer.

The procedure also can be performed by adding appropriate amounts of the substrate media to the sample, mixing thoroughly, and dispensing into five 20-mL or ten 10-mL sterile tubes. Incubate as stated for multiple-tube procedure.

* Colilert® and Colilert 18® and Colisure™ for multi-tube, P/A, and tray formats available from IDEXX Laboratories, Inc., Westbrook, ME.
† Quanti-Tray® or Quanti-Tray®/2000, available from IDEXX Laboratories, Inc., Westbrook, ME.

TABLE 9223:I. COLOR CHANGES FOR VARIOUS MEDIA

Substrate	Total Coliform Positive	*E. coli* Positive	Negative Result
ONPG-MUG	Yellow	Blue fluorescence	Colorless/no fluorescence
CPRG-MUG	Red or magenta	Blue fluorescence	Yellow/no fluorescence

b. Multi-well procedure: The multi-well procedure is performed with sterilized disposable packets. Add enzyme substrate to a 100-mL sample in a container, shake vigorously, and pour into tray. The tray sealer dispenses the sample into the wells and seals the package. Incubate at 35 ± 0.5°C for period specified by substrate manufacturer. The MPN value is obtained from the table provided by the manufacturer.

c. Presence-absence procedure (P/A): Aseptically add preweighed enzyme medium to 100-mL sample in a sterile, transparent, nonfluorescent borosilicate glass or equivalent bottle or container. Optionally, add the enzyme substrate to a 100-mL sample in a sterile nonfluorescent container that is purchased commercially. Aseptically cap and mix thoroughly to dissolve. Incubate as specified in manufacturer's instructions.

3. Interpretation

a. Total coliform bacteria: After the minimum proper incubation period, examine tubes or containers for the appropriate color change (Table 9223:I). ONPG is hydrolyzed by the bacterial enzyme to yield a yellow color. CPRG is hydrolyzed by the bacterial enzyme to yield a red or magenta color. If the color response is not uniform throughout the sample, mix by inversion before reading. Read manufacturer's instructions for interpretation guidelines. Some manufacturers suggest comparing sample against a color comparator available through the manufacturer. Samples are negative for total coliforms if no color is observed in ONPG tests or if the tube is yellow when CPRG is used. If a chromogenic response is questionable after 18 or 24 h for ONPG, incubate up to an additional 4 h. If response is negative after 24 h for CPRG, incubate up to an additional 24 h. If the chromogen intensifies, the sample is total-coliform positive; if it does not, the sample is negative.

b. Escherichia coli: Examine positive total coliform tubes or containers for fluorescence using a long-wavelength (365-nm) ultraviolet lamp (6-W bulb). Compare each tube against the reference comparator available from a commercial source of the substrate. The presence of fluorescence is a positive test for *E. coli*. If fluorescence is questionable, incubate for an additional 4 h for ONPG tests and up to an additional 24 h for CPRG tests; intensified fluorescence is a positive test result.

4. Reporting

If performing an MPN procedure, calculate the MPN value for total coliforms and *E. coli* from the number of positive tubes as described in Section 9221C. If using the presence-absence procedure, report results as total coliform and *E. coli* present or absent in 100-mL sample.

5. Quality Control

Test each lot of media purchased for performance by inoculation with three control bacteria: *Escherichia coli,* a total coliform other than *E. coli* (e.g., *Enterobacter cloacae*), and a noncoliform. Also add a sterile water control. If the sterile water control exhibits faint fluorescence or faint positive coliform result, discard and use a new batch of substrate. Avoid using a heavy inoculum. If *Pseudomonas* is used as the representative noncoliform, select a nonfluorescent species. Incubate these controls at 35 ± 0.5°C as indicated above. Read and record results. Other quality-control guidelines are included in Section 9020.

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Coliforms, Total and *E. coli*

DOC316.53.01213

m-ColiBlue24 Broth PourRite Ampules[1]

Method 10029

Membrane Filtration

Scope and application: For potable water, nonpotable water, recreation water and wastewater.

[1] USEPA approved.

Test preparation

Before starting

Let the media in PourRite ampules increase to room temperature before the ampule is opened.
Set the temperature of the incubator to 35 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Wash hands thoroughly with soap and water.
Use a germicidal cloth, bactericidal spray, weak bleach solution or weak iodine solution to clean the work area.
Make sure that all of the materials that come in contact with samples are sterile.
During filtration, remove the vacuum as soon as the funnel is empty so that the membrane filter does not become dry.
As an alternative to the filter assembly with flask, use a sterile, disposable filter unit.

Items to collect

Description	Quantity
Broth ampule, m-ColiBlue24	1
Sterile buffered dilution water	1
Membrane filter, 0.45 micron	1
Petri dish with absorbent pad, 47-mm	1
Filtration apparatus with aspirator or pump	1
Forceps, sterilized	1
Incubator	1
Microscope, low-power	1
Pipet(s) for dilution or for sample volumes less than 100 mL, if necessary	1

Refer to Consumables and replacement items on page 6 for order information.

Sample collection

- Use a sterile glass or plastic container such as a Whirl-Pak bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inner surfaces of the container. Do not rinse the containers before use.
- To collect a potable water sample from a faucet, spigot, hydrant or pump, let the water flow at a moderate rate for 2–3 minutes. Remove the screens or aerators. Do not use faucets or spigots that have a bad seal or that show a leak between components.
- To collect a non-potable sample from a river, lake or reservoir, hold the container below the water surface, then remove the cap. As an alternative, remove the cap and push the container, mouth down, below the water surface to prevent the collection of surface scum. Put the mouth of the container into the current. Fully fill the container below the water surface.
- Collect a minimum of 100 mL of sample. Keep a minimum of 2.5 cm (1 inch) of air space in the container.
- Write the sample information on the container and start the analysis as soon as possible.
- If immediate analysis is not possible, keep the sample at or below 10 °C (50 °F) for a maximum of 8 hours. Do not let the sample freeze.

Sample volumes

Use a sample volume that is applicable to the sample type. For samples with a low level of bacteria such as finished, potable water, use 100 mL of sample. Use less sample for non-potable water or water that contains more bacteria.

When the approximate bacteria level is unknown, analyze three different sample volumes. Use the results from the sample volume that shows approximately 20 to 200 colonies for each membrane filter.

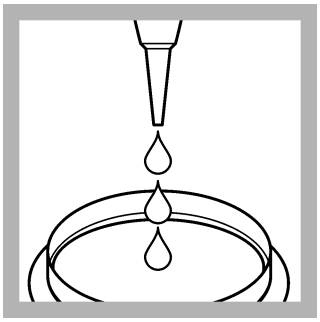
When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile buffered dilution water to the filter funnel before the vacuum is applied. The additional dilution water helps to apply the bacteria equally across the membrane filter.

Sample dilution

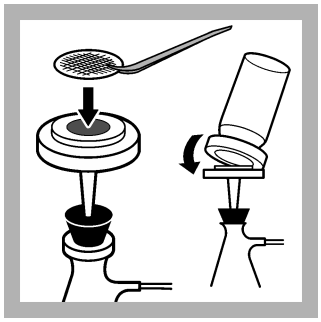
Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

1. Wash hands thoroughly with soap and water.
2. Invert the sample container for 30 seconds (approximately 25 times).
3. Open a bottle of sterile buffered dilution water.
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 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
8. If necessary, continue to dilute the sample.

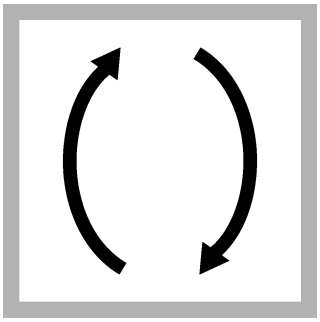
Membrane filtration test procedure



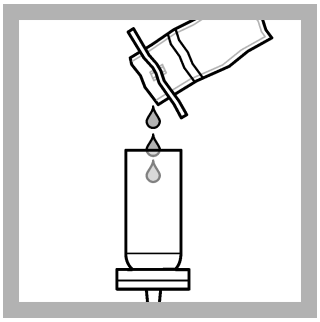
1. Invert one m-ColiBlue24 broth ampule 2 to 3 times. Open the ampule. Lift the lid of a petri dish and carefully pour the contents equally on the absorbent pad.



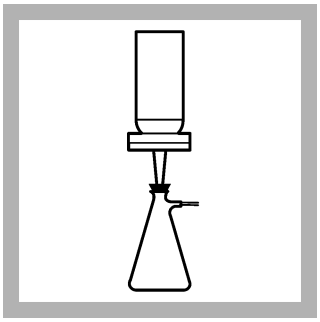
2. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.



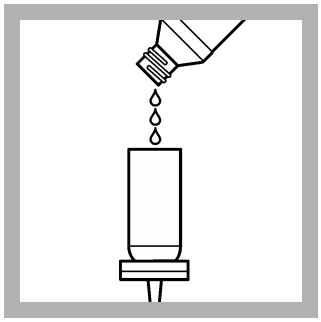
3. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.



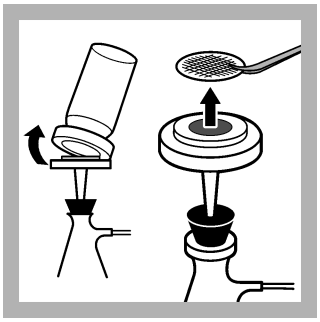
4. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.



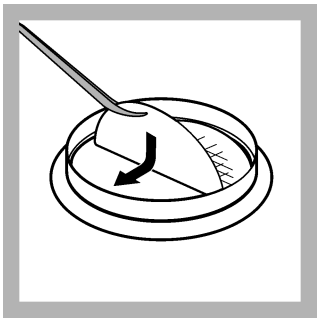
5. Apply the vacuum until the funnel is empty. Stop the vacuum.



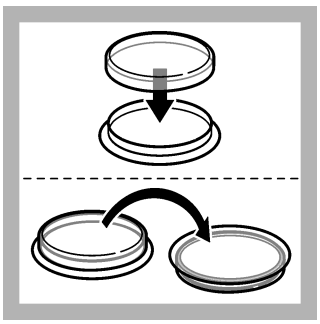
6. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.



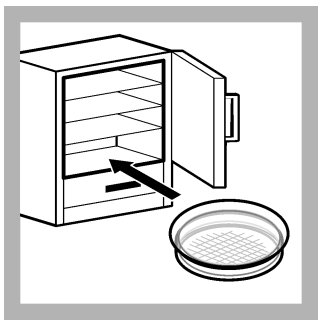
7. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.



8. Put the membrane filter on the absorbent pad. Let the membrane filter bend and fall equally across the absorbent pad to make sure that air bubbles are not caught below the filter.



9. Put the lid on the petri dish and invert the petri dish.



10. Incubate the inverted petri dish at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 hours.

11. Remove the petri dish from the incubator. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter. Refer to Interpret and report the coliform results on page 5.

Optional testing of red colonies

The m-ColiBlue24 Broth is made so that coliforms other than E. coli form red colonies. The percentage of red colonies that are false positives (non-coliforms) is equivalent to the

percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms). So, a confirmation procedure is not necessary.

Some varieties of the non-coliform bacteria *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. can form on m-ColiBlue24 Broth and form red colonies. Use the oxidase method to quickly identify between these bacteria and total coliforms. *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If the sample has high levels of interfering bacteria, do an oxidase method to identify which of the red colonies are total coliforms.

Two oxidase methods follow. Count the red and blue colonies on the m-ColiBlue24 Broth membrane filter before the oxidase method is started.

Oxidase method 1

Use this method to easily and quickly analyze membrane filters that have numerous colonies. Use this method after 24 hours of incubation on m-ColiBlue24 Broth.

Research[1] shows that the oxidase method cannot be done on media where acidification of the media occurs during bacterial growth. The m-ColiBlue24 Broth is made so that acidification of the medium does not occur. As a result, the method can analyze many colonies at the same time for their oxidase reaction.

1. Remove the lid from the petri dish.
2. Invert the lid.
3. Put the lid on the bench top.
4. Use a dropper to put 0.5 mL of Difco SpotTest Oxidase Reagent in the center of the inverted lid.
5. Use sterile forceps to move the membrane filter from the pad in the petri dish to the inverted lid.

   After 10 to 15 seconds, the filter absorbs the Difco SpotTest Oxidase Reagent. The oxidase-positive colonies change from red to purple. This purple color shows in the colony or adjacent to the colony. Oxidase-negative colonies stay red.
6. After the initial 10 to 15 second reaction time, count the red colonies that are purple in 30 seconds or less. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter.

   For easy colony counting, put a spare lid on the petri dish lid. Use a felt-tip pen to put a mark on each purple colony. After 30 seconds, count the marks.

   Note: *The red oxidase-negative colonies start to change to a purple color after 30 seconds after the initial 10 to 15 second reaction time.*

Bacteria are not killed with this method, so colonies can be selected for streaking and for more testing.

Colonies that are blue after the initial 24-hour incubation are almost always *E. coli*. So, confirmation with the oxidase method is not necessary.

Oxidase method 2

This method is the official oxidase test 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 on Tryptic Soy Agar.
2. Incubate the Tryptic Soy Agar plates at 35 °C (95 °F) for 18 to 24 hours or until the isolated colonies form.

[1] 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.

3. Soak a piece of filter paper with Difco SpotTest Oxidase Reagent. This reagent has a stabilized solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride.

   Note: *As an alternative, use a dropper to add oxidase reagent directly on colonies on Tryptic Soy Agar. Oxidase-positive colonies change from pink to purple.*

4. Use a sterile nichrome inoculating needle to move 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 cause false-positive results. Wooden applicator sticks work well.*

   Oxidase-negative colonies will not react with the reagent, but oxidase-positive colonies cause the reagent to change to dark purple within 10 seconds.

5. Count the oxidase-negative colonies as total coliform bacteria.

Interpret and report the coliform results

Report the coliform density as the number of colonies in 100 mL of sample. For total coliforms, use a sample volume that gives 20–80 coliform colonies on the membrane filter. For fecal coliforms, use a sample volume that gives 20–60 fecal coliform colonies on the membrane filter.

If there are more than 200 colonies, dilute the sample and use the diluted sample in the test procedure. Use the sample volume before dilution in the coliform density determination.

1. Use the microscope to look at the colonies on the membrane filter. Count the number of isolated coliform colonies.
2. Determine the coliform density as follows:

Membrane filter(s)	Coliform density determination
One membrane filter	Coliform colonies in 100 mL = Coliform colonies counted ÷ mL sample × 100 *Example: 50 coliform colonies were counted. The sample volume was 20 mL. The coliform density is 50 ÷ 20 mL × 100 = 250 coliforms in 100 mL of sample.*
Multiple filters, dilutions or duplicates for each sample	Average coliform colonies in 100 mL = Sum of coliform colonies in all samples ÷ sum of mL sample × 100 *Example: Two 50-mL samples gave 5 colonies on one filter and 9 colonies on another filter. The coliform density is (5 + 9) ÷ (50 + 50) × 100 = 14 coliforms in 100 mL of sample.*

3. If colonies are not isolated or if there are more than 200 colonies of all types:
   a. Report the results as "Confluent growth with or without coliforms" when the bacteria grows together across some or all of the membrane filter.
   b. Do the test procedure again with half the sample volume. If the total number of colonies (coliforms plus non-coliforms) is more than 200 for each membrane or the colonies are not isolated, report the results as "Too numerous to count" (TNTC).
   c. Do the test procedure again with a dilution that gives approximately 50 coliform colonies and not more than 200 colonies of all types.

Controls for coliform bacteria tests

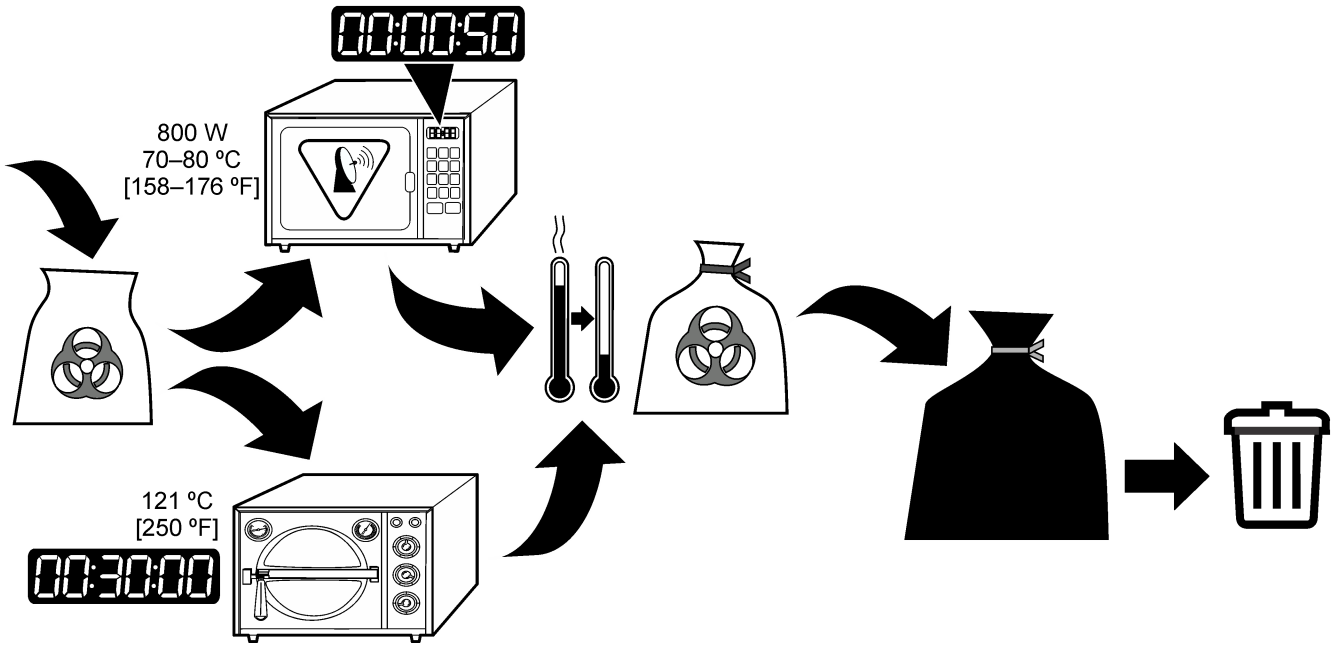
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive control.

Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to Figure 1 and the information that follows.

- Microwave—Add 1–2 mL of hypochlorite (bleach) solution to each test container. If a container has a lid, do not close it too tightly. Put the container in the microwave at 70–80 °C (158–176 °F) for 50 seconds. Wait 10 to 15 minutes. Pour the liquid down the drain.
- Autoclave—Put the used test containers in a contaminated items bag or biohazard bag to prevent leaks. Do not seal the bag. Put the bag in the autoclave at 121 °C (250 °F) for 30 minutes at 1.0 bar (15 psi) of pressure. When the bag is cool, seal it and put it into a garbage bag. Make sure to tie the garbage bag tightly.

Figure 1 Bacteria disposal



Summary of method

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages, surface water, well water, groundwater, waste water, recreational waters and process water for ultrapure, chemical processing and pharmaceutical applications.

Count all of the red and blue colonies as total coliforms. Count all of the blue colonies as *E. coli*. Blue colonies can be blue to purple.

Note: *Sometimes only the center of a colony shows color. Count a colony with any red color as red. Count a colony with any blue as a blue colony. Red colonies can be different in color intensity.*

The membrane filtration procedure is used for samples that are low in turbidity and have low bacteria counts. The sample is poured through a membrane filter. The bacteria in the sample stays on the membrane filter. The membrane filter is moved to a petri dish that contains a nutritional broth or agar. During incubation, the bacteria grow and form colonies on the membrane filter. After incubation, the filter is examined with a microscope for bacteria colonies.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
m-ColiBlue24® broth ampules, glass	1	20/pkg	2608420
m-ColiBlue24® broth ampules, plastic	1	50/pkg	2608450

Consumables and replacement items (continued)

Description	Quantity/test	Unit	Item no.
m-ColiBlue24®, prepared agar plates	1	15/pkg	2805215
Dilution water, buffered, 99 mL, sterile[2]	1	25/pkg	1430598

Required apparatus

Description	Unit	Item no.
Ampule breaker, PourRite™	each	2484600
Membrane filter holder, magnetic, 300-mL funnel	each	1352900
Filter pump, aspirator	each	213100
Flask, filtering, glass, 1000 mL	each	54653
Forceps, stainless steel	each	2141100
Membrane filter, 0.45 micron, 47 mm diameter, sterile	200/pkg	1353001
Membrane filter, 0.45 micron, 47 mm diameter, sterile EO (ethylene oxide)	150/pkg	2936100
Microscope, compound	each	2947050
Petri dish with absorbent pad, for 47-mm membrane filters, sterile	100/pkg	1471799
Petri dish with absorbent pad, for 47-mm membrane filters, sterile EO (ethylene oxide)	150/pkg	25248000
Stopper, rubber, size 8, for filtration assembly	6/pkg	211908
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Tubing, rubber, 7.9 mm (5/16-in.) inside diameter	3.66 m (12 ft)	56019

Incubators

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Portable incubator with 12 VDC power socket	each	2569900
AC power supply for portable incubator, 110–240 VAC	each	2968100
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Portable incubator rack, general purpose/petri dish	each	2580502

Sample collection

Description	Unit	Item no.
Sampling bags, Whirl-Pak® with dechlorinating reagent, 177 mL	100/pkg	2075333
Sampling bags, Whirl-Pak without dechlorinating reagent, 207 mL	100/pkg	2233199
Sampling bottles, sterilized, with dechlorinating agent, 100-mL sample	100/pkg	8888006
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	12/pkg	2495012
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	50/pkg	2495050
Sample transport kit, includes 100 sample bags with dechlorinating agent, refrigerant pack, rack and 9-L cooler	each	2568700

[2] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Optional reagents and apparatus

Description	Unit	Item no.
m-ColiBlue24® Broth, glass bottle	100 mL	2608442
Disposable filter funnels with membrane filters, sterile	150/pkg	2586300
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pipet, serological, 2 mL, sterile, glass	35/pkg	2093136
Pipet filler, safety bulb	each	1465100
Support base for disposable filter funnels	each	2586201
Vacuum pump, hand-operated	each	1428300

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
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10/2018, Edition 11

Coliforms, Total, Fecal and *E. coli*

DOC316.53.01224

m-Endo Broth Ampule[1]

Method 8074

Membrane Filtration

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.

Test preparation

Before starting

Let the media in PourRite ampules increase to room temperature before the ampule is opened.
Set the temperature of the incubator to 35 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Potable water must have no coliform bacteria. Do not dilute potable water samples.
Wash hands thoroughly with soap and water.
Use a germicidal cloth, bactericidal spray, weak bleach solution or weak iodine solution to clean the work area.
Make sure that all of the materials that come in contact with samples are sterile.
During filtration, remove the vacuum as soon as the funnel is empty so that the membrane filter does not become dry.
As an alternative to the filter assembly with flask, use a sterile, disposable filter unit.
As an alternative to the m-Endo broth, use m-Endo agar plates.

Items to collect

Description	Quantity
Broth ampule, m-Endo	1
Confirmation media	varies
Sterile buffered dilution water	varies
Membrane filter, 0.45 micron	1
Petri dish with absorbent pad, 47-mm	1
Filtration apparatus with aspirator or pump	1
Forceps, sterilized	1
Incubator	1
Microscope, low-power	1
Pipet(s) for dilution or for sample volumes less than 100 mL, if necessary	1

Refer to Consumables and replacement items on page 10 for order information.

Sample collection

- Use a sterile glass or plastic container such as a Whirl-Pak bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inner surfaces of the container. Do not rinse the containers before use.
- To collect a potable water sample from a faucet, spigot, hydrant or pump, let the water flow at a moderate rate for 2–3 minutes. Remove the screens or aerators. Do not use faucets or spigots that have a bad seal or that show a leak between components.
- To collect a non-potable sample from a river, lake or reservoir, hold the container below the water surface, then remove the cap. As an alternative, remove the cap and push the container, mouth down, below the water surface to prevent the collection of surface scum. Put the mouth of the container into the current. Fully fill the container below the water surface.
- Collect a minimum of 100 mL of sample. Keep a minimum of 2.5 cm (1 inch) of air space in the container.
- Write the sample information on the container and start the analysis as soon as possible.
- If immediate analysis is not possible, keep the sample at or below 10 °C (50 °F) for a maximum of 8 hours. Do not let the sample freeze.

Sample volumes

Use a sample volume that is applicable to the sample type. For samples with a low level of bacteria such as finished, potable water, use 100 mL of sample. Use less sample for non-potable water or water that contains more bacteria.

When the approximate bacteria level is unknown, analyze three different sample volumes. Use the results from the sample volume that shows approximately 20 to 200 colonies for each membrane filter.

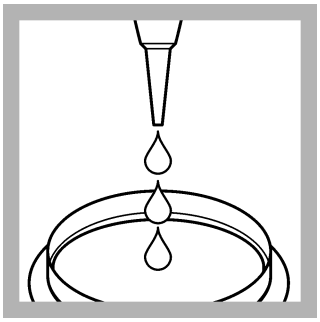
When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile buffered dilution water to the filter funnel before the vacuum is applied. The additional dilution water helps to apply the bacteria equally across the membrane filter.

Sample dilution

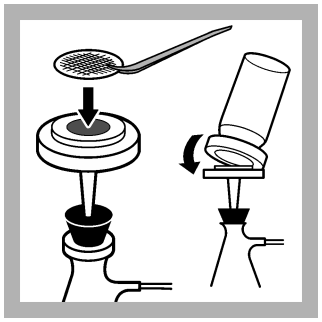
Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

1. Wash hands thoroughly with soap and water.
2. Invert the sample container for 30 seconds (approximately 25 times).
3. Open a bottle of sterile buffered dilution water.
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 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
8. If necessary, continue to dilute the sample.

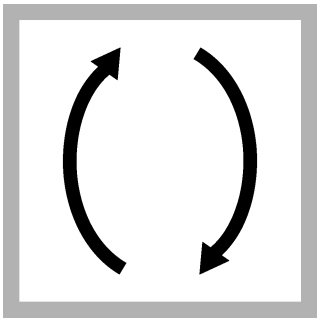
Presumptive test for total coliforms



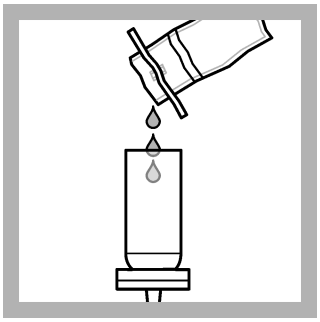
1. Invert one m-Endo broth ampule 2 to 3 times. Open the ampule. Lift the lid of a petri dish and carefully pour the contents equally on the absorbent pad.



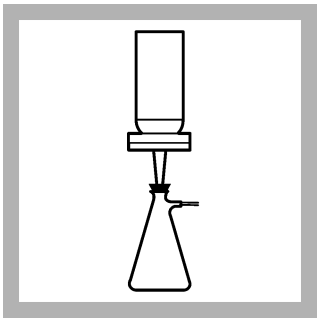
2. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.



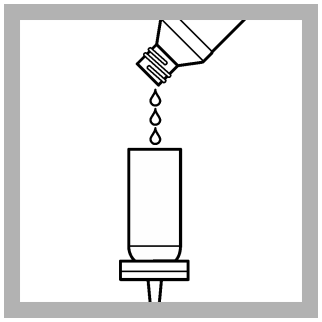
3. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.



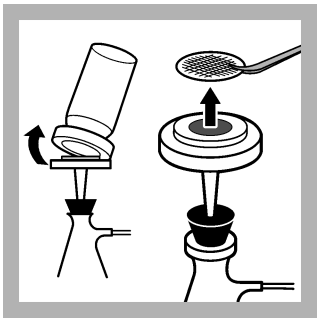
4. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.



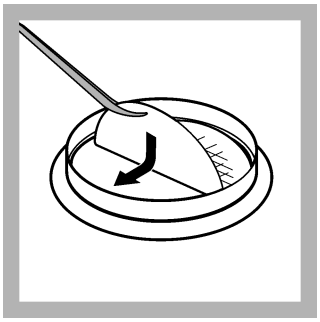
5. Apply the vacuum until the funnel is empty. Stop the vacuum.



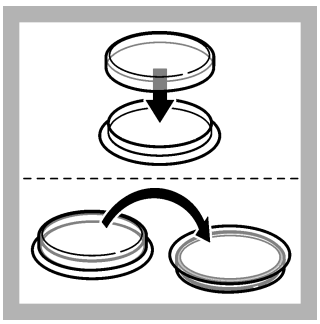
6. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.



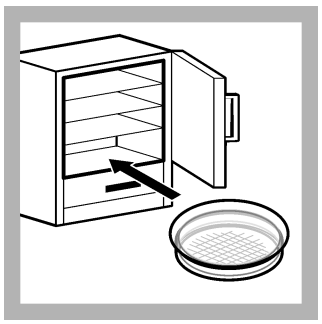
7. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.



8. Put the membrane filter on the absorbent pad. Let the membrane filter bend and fall equally across the absorbent pad to make sure that air bubbles are not caught below the filter.



9. Put the lid on the petri dish and invert the petri dish.



10. Incubate the inverted petri dish at 35 ± 0.5 °C (95 ± 0.9 °F) for 22–24 hours.

About confirmation of total coliforms

For potable water samples, do the confirmation procedure on typical colonies to make sure that they are coliforms. Confirm sheen colonies to a maximum of five. Move growth from each colony to inoculate parallel tubes of Lauryl Tryptose (LT) single-strength (SS) broth and Brilliant Green Bile (BGB) broth. Growth and gas production in the two tubes makes sure that the organisms are coliforms. Most Probable Number (MPN) coliform tubes are recommended for this procedure.

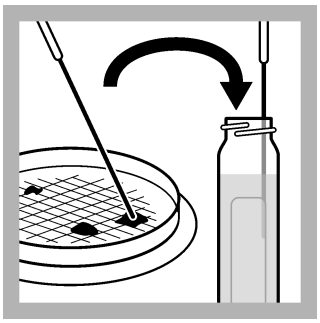
Use the swabbing technique for fecal coliforms or *E. coli* as follows:

- To determine only if total coliforms are in or not in the sample
- To inoculate EC or EC/MUG media

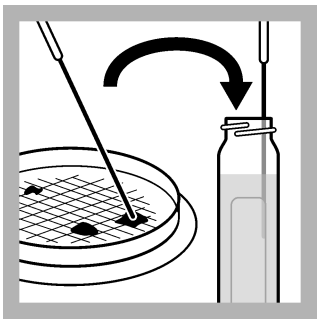
Inoculate in the sequence that follows:

1. EC or EC/MUG media
2. Lauryl Tryptose (LT) single-strength broth
3. Brilliant Green Bile (BGB) broth

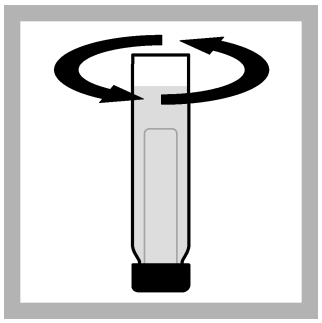
Confirmation test of total coliforms (LT and BGB)



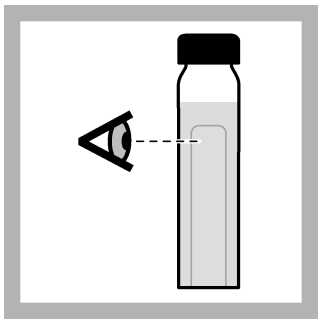
1. Touch a sterilized inoculating needle or a sterile disposable needle to the coliform (sheen) colony growth. Put the needle in a Lauryl Tryptose broth tube.



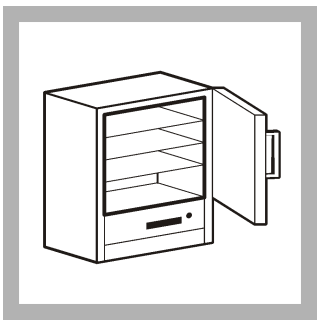
2. Touch the sterilized inoculating needle again to the same coliform (sheen) colony growth. Put the needle in a Brilliant Green Bile (BGB) broth tube.



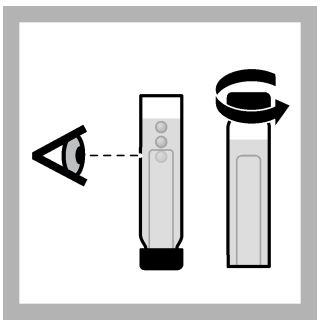
3. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



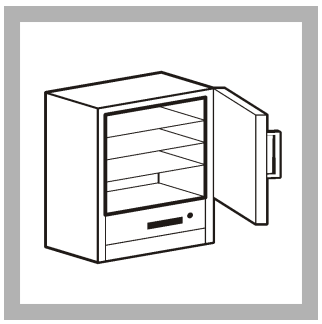
4. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



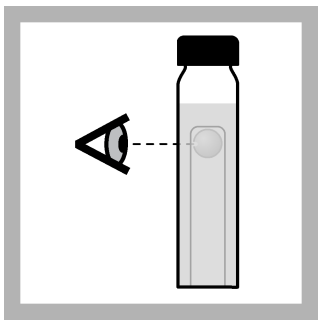
5. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 1 hour.
Bubbles that form in the inner vials during the first hour are not from bacteria.



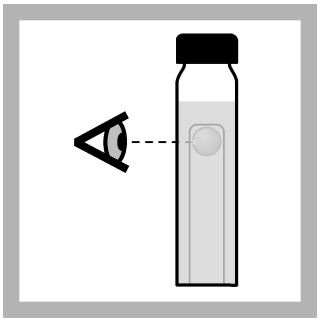
6. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



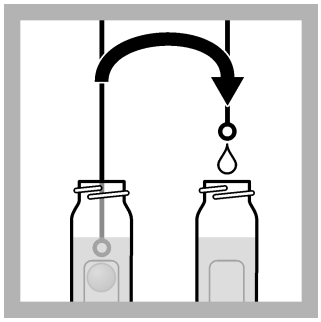
7. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 ± 2 hours.
Note: *It is necessary to keep the tubes in a vertical position for the remainder of the test.*



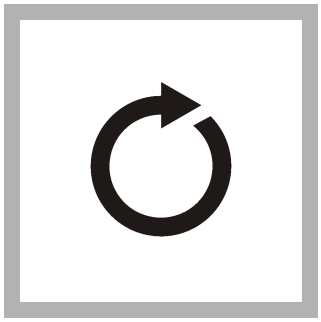
8. After 24 ± 2 hours, remove the samples from the incubator. Tap each tube gently and examine the inner vials for gas.
If the broth is cloudy and the inner vials contain gas bubbles, coliform bacteria are likely in the sample. Any gas that shows is an indication of coliform bacteria.
If no gas can be seen, put the tubes in the incubator for 24 ± 2 hours (48 ± 3 hours total) and examine the tubes again.



9. After 48 ± 3 hours, gently tap each tube and examine the inner vials for gas. If the inner vial contains gas bubbles, the test is positive for total coliform bacteria. If none of the tubes contain gas, then the test is negative for total coliform bacteria.

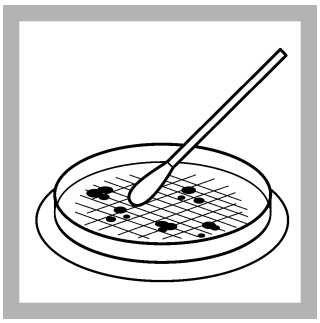


10. Confirm positive results. If growth and gas occur in the Lauryl Tryptose broth tube but not in the Brilliant Green Bile (BGB) broth tube, inoculate another Brilliant Green Bile (BGB) broth tube from the gas-positive Lauryl Tryptose broth tube.

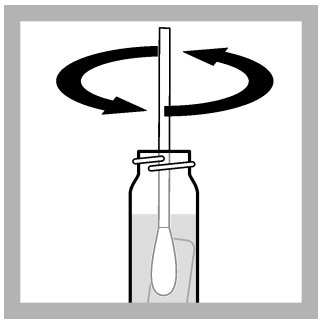


11. Do steps 3–9 again on the Brilliant Green Bile (BGB) broth tube. If growth and gas occur within 48 ± 3 hours, the colony is confirmed as coliform.

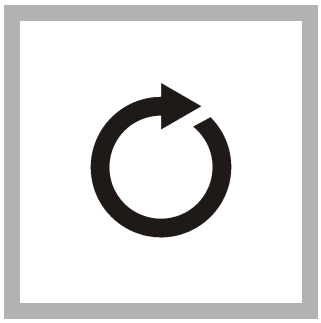
Confirmation test for fecal coliforms (EC Medium)



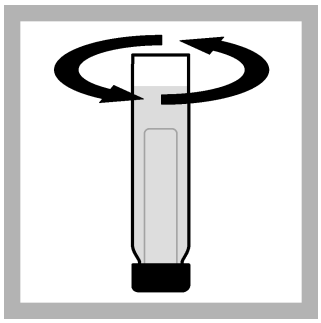
1. Use a sterile cotton swab or inoculating loop to touch all of the surface of the membrane filter that is positive for total coliforms.



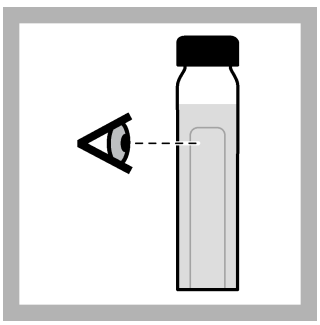
2. Swirl the cotton swab or inoculating loop in an EC Medium Broth tube to move the colonies collected from the filter to the tube.



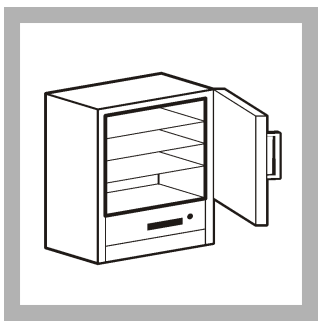
3. Do steps 1–2 again for each test to be verified. Use one broth tube for each test. Use the same cottom swab.



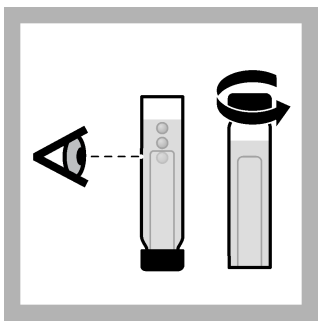
4. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



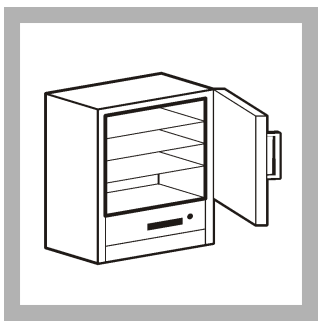
5. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



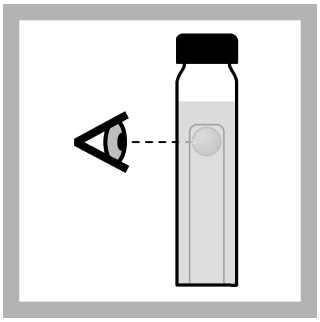
6. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



7. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



8. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 ± 2 hours.



9. After 24 ± 2 hours, remove the samples from the incubator. Gently tap each tube 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, then the test is negative for fecal coliform bacteria.

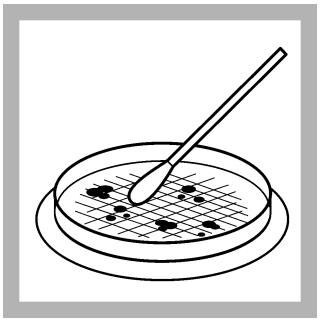
Confirmation of *E. coli* (EC or EC/MUG)

⚠CAUTION

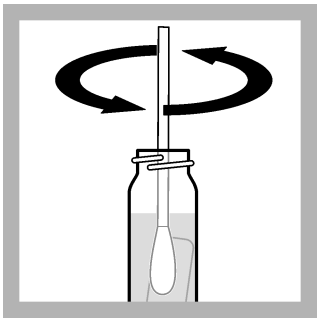
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

When the nutritional media contains MUG, use a long-wave (e.g., 365 nm) UV lamp to confirm the presence of *E. coli*. The sample will fluoresce if *E. coli* is in the sample. No additional confirmation procedure is necessary.

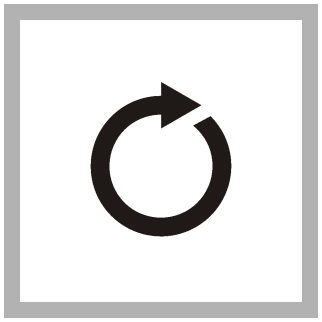
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



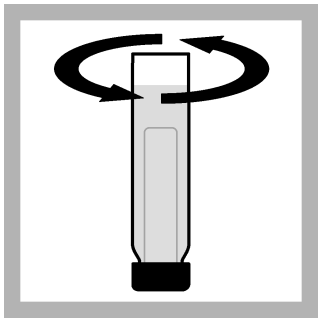
1. Use a sterile cotton swab or inoculating loop to touch all of the surface of the membrane filter that is positive for total coliforms.



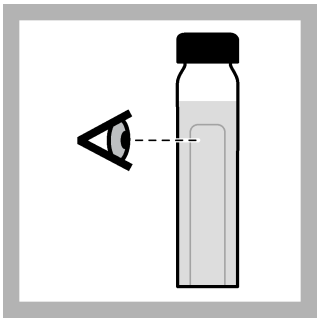
2. Swirl the cotton swab or inoculating loop in an EC/MUG Broth tube to move the colonies collected from the filter to the tube.



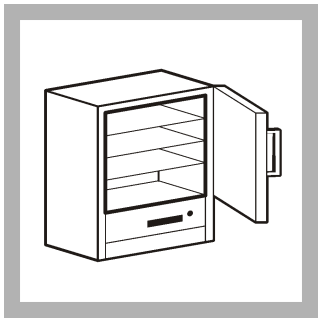
3. Do steps 1–2 again for each test to be verified. Use one broth tube for each test. Use the same cottom swab.



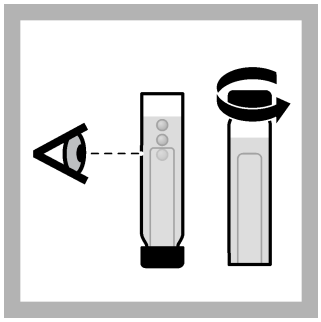
4. Invert the tubes to remove air from the inner vials. Gently swirl, if necessary.



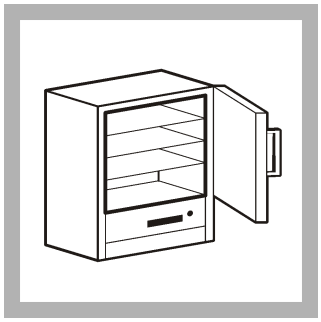
5. Examine the tubes to make sure that the inner vial (if Durham tubes are used) is full of liquid with no air bubbles.



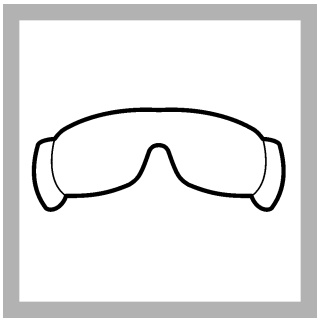
6. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 1 hour. Bubbles that form in the inner vials during the first hour are not from bacteria.



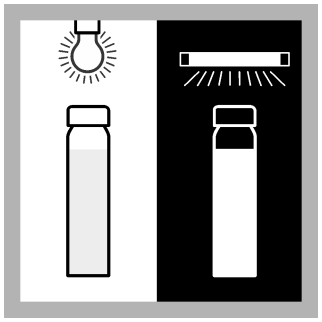
7. After 1 hour, invert the tubes to remove air from the inner vials. Make sure that there are no bubbles and keep the tubes in a vertical position. Loosen the caps only a little, then put the tubes in the incubator.



8. Incubate the inoculated confirmation media at 44.5 ± 0.2 °C (112.1 ± 0.4 °F) for 24 ± 2 hours.



9. Put on UV safety goggles



10. Apply UV light to the incubated sample that contains MUG broth with a long-wave UV lamp. Examine the tubes in a dark area. Look at the tube 90° from the UV light. Compare the fluorescence of the sample tubes to a tube that contains a known *E. coli* positive confirmation. If the sample fluoresces, *E. coli* bacteria are in the sample. If the sample does not fluoresce, the test is negative for *E. coli*.

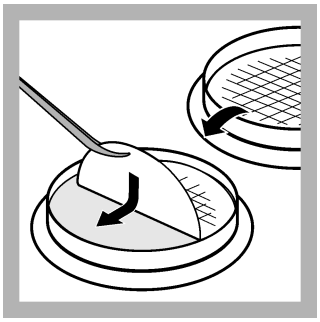
Confirmation of E. coli (Nutrient Agar/MUG)

⚠CAUTION

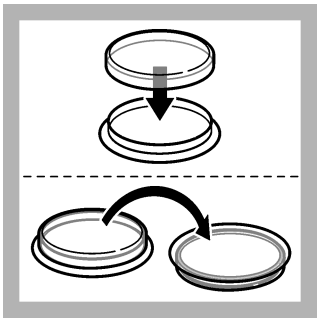
Ultraviolet (UV) light exposure hazard. Exposure to UV light can cause eye and skin damage. Protect eyes and skin from direct exposure to UV light.

When the nutritional media contains MUG, use a long-wave (e.g., 365 nm) UV lamp to confirm the presence of *E. coli*. The sample will fluoresce if *E. coli* is in the sample. No additional confirmation procedure is necessary.

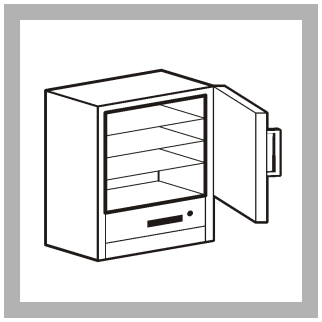
Note: *The sample container can fluoresce slightly. To help with fluorescence detection, use an E. coli Fluorescence Standard. Compare the fluorescence from the sample and the standard.*



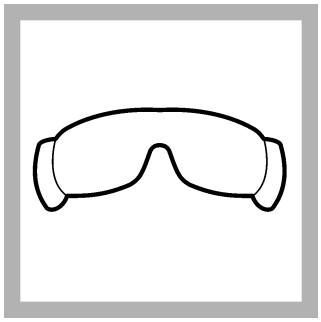
1. Use sterile forceps to put the membrane filter that is positive for total coliforms on the prepared NA/MUG agar plate. Let the membrane filter bend and fall equally across the agar to make sure that air bubbles are not caught below the filter.



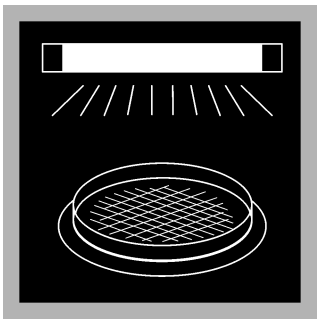
2. Put the lid on the petri dish and invert the petri dish.



3. Incubate the inoculated confirmation media at 35 ± 0.5 °C (95 ± 0.9 °F) for 4 hours.



4. Put on UV safety goggles



5. Remove the lid from the petri dish. Apply UV light to the colonies in a dark area. If the colonies fluoresce, *E. coli* bacteria are in the sample.
If the colonies do not fluoresce, the test is negative for *E. coli*.

Interpret and report the coliform results

Report the coliform density as the number of colonies in 100 mL of sample. For total coliforms, use a sample volume that gives 20–80 coliform colonies on the membrane filter. For fecal coliforms, use a sample volume that gives 20–60 fecal coliform colonies on the membrane filter.

If there are more than 200 colonies, dilute the sample and use the diluted sample in the test procedure. Use the sample volume before dilution in the coliform density determination.

1. Use the microscope to look at the colonies on the membrane filter. Count the number of isolated coliform colonies.
2. Determine the coliform density as follows:

Membrane filter(s)	Coliform density determination
One membrane filter	Coliform colonies in 100 mL = Coliform colonies counted ÷ mL sample × 100 *Example: 50 coliform colonies were counted. The sample volume was 20 mL. The coliform density is 50 ÷ 20 mL × 100 = 250 coliforms in 100 mL of sample.*
Multiple filters, dilutions or duplicates for each sample	Average coliform colonies in 100 mL = Sum of coliform colonies in all samples ÷ sum of mL sample × 100 *Example: Two 50-mL samples gave 5 colonies on one filter and 9 colonies on another filter. The coliform density is (5 + 9) ÷ (50 + 50) × 100 = 14 coliforms in 100 mL of sample.*

3. If colonies are not isolated or if there are more than 200 colonies of all types:
   a. Report the results as “Confluent growth with or without coliforms” when the bacteria grows together across some or all of the membrane filter.
   b. Do the test procedure again with half the sample volume. If the total number of colonies (coliforms plus non-coliforms) is more than 200 for each membrane or the colonies are not isolated, report the results as “Too numerous to count” (TNTC).
   c. Do the test procedure again with a dilution that gives approximately 50 coliform colonies and not more than 200 colonies of all types.

Controls for coliform bacteria tests

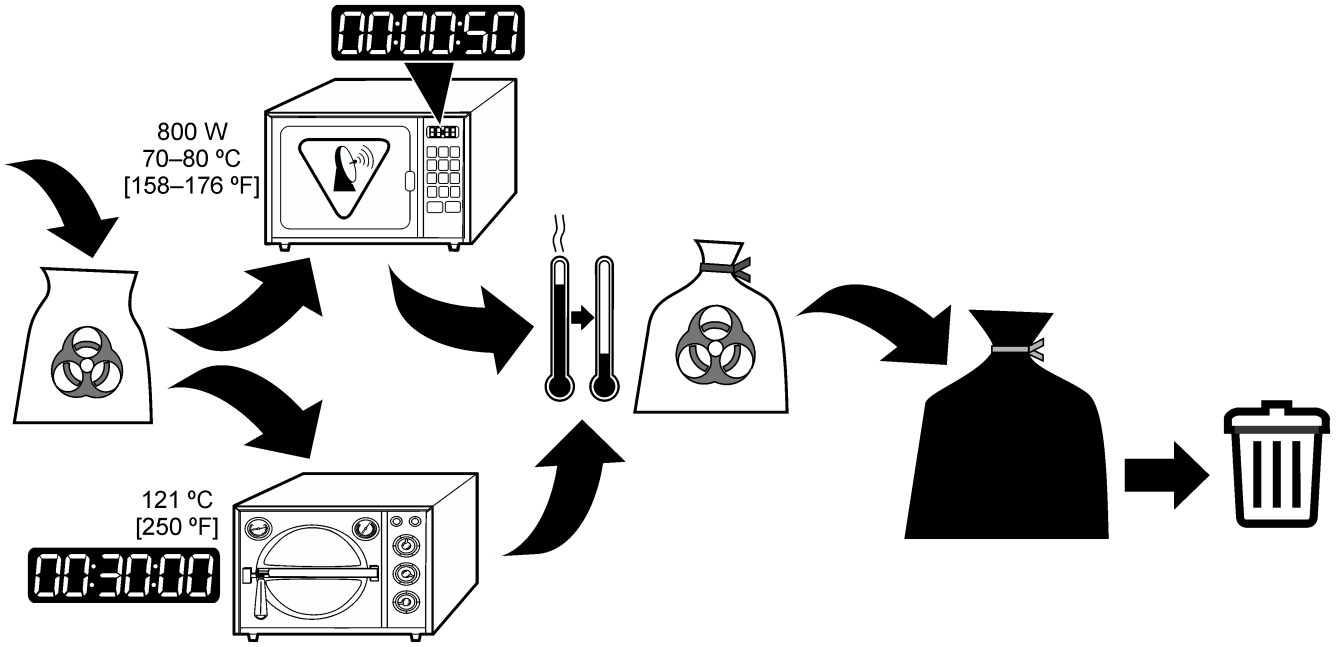
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive control.

Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to Figure 1 and the information that follows.

- Microwave—Add 1–2 mL of hypochlorite (bleach) solution to each test container. If a container has a lid, do not close it too tightly. Put the container in the microwave at 70–80 °C (158–176 °F) for 50 seconds. Wait 10 to 15 minutes. Pour the liquid down the drain.
- Autoclave—Put the used test containers in a contaminated items bag or biohazard bag to prevent leaks. Do not seal the bag. Put the bag in the autoclave at 121 °C (250 °F) for 30 minutes at 1.0 bar (15 psi) of pressure. When the bag is cool, seal it and put it into a garbage bag. Make sure to tie the garbage bag tightly.

Figure 1 Bacteria disposal



Summary of method

Coliforms ferment lactose in the medium and form an acid-aldehyde complex. The complex mixes with Schiff's Reagent (also in the medium) to form an iridescent green coating above the colonies. When magnified 10x to 15x, coliforms show as dark red colonies with a greenish-gold sheen.

The membrane filtration procedure is used for samples that are low in turbidity and have low bacteria counts. The sample is poured through a membrane filter. The bacteria in the sample stays on the membrane filter. The membrane filter is moved to a petri dish that contains a nutritional broth or agar. During incubation, the bacteria grow and form colonies on the membrane filter. After incubation, the filter is examined with a microscope for bacteria colonies.

Consumables and replacement items

Presumptive for total coliforms (BGB and LT)

Required reagents

Description	Quantity/test	Unit	Item no.
m-Endo broth ampules, plastic	1	50/pkg	2373550
m-Endo broth PourRite™ ampules, glass (for total coliform presumptive)	1	20/pkg	2373520
m-Endo, prepared agar plates	1	15/pkg	2811615
Dilution water, buffered, 99 mL, sterile[1]	1	25/pkg	1430598

Required apparatus

Description	Unit	Item no.
Ampule breaker, PourRite™	each	2484600
Membrane filter holder, magnetic, 300-mL funnel	each	1352900
Filter pump, aspirator	each	213100

[1] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Required apparatus (continued)

Description	Unit	Item no.
Flask, filtering, glass, 1000 mL	each	54653
Forceps, stainless steel	each	2141100
Membrane filter, 0.45 micron, 47 mm diameter, sterile	200/pkg	1353001
Membrane filter, 0.45 micron, 47 mm diameter, sterile EO (ethylene oxide)	150/pkg	2936100
Microscope, compound	each	2947050
Petri dish with absorbent pad, for 47-mm membrane filters, sterile	100/pkg	1471799
Petri dish with absorbent pad, for 47-mm membrane filters, sterile EO (ethylene oxide)	150/pkg	25248000
Stopper, rubber, size 8, for filtration assembly	6/pkg	211908
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Tubing, rubber, 7.9 mm (5/16-in.) inside diameter	3.66 m (12 ft)	56019

Incubators

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Portable incubator with 12 VDC power socket	each	2569900
AC power supply for portable incubator, 110–240 VAC	each	2968100
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Portable incubator rack, general purpose/petri dish	each	2580502

Sample collection

Description	Unit	Item no.
Sampling bags, Whirl-Pak® with dechlorinating reagent, 177 mL	100/pkg	2075333
Sampling bags, Whirl-Pak without dechlorinating reagent, 207 mL	100/pkg	2233199
Sampling bottles, sterilized, with dechlorinating agent, 100-mL sample	100/pkg	8888006
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	12/pkg	2495012
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	50/pkg	2495050
Sample transport kit, includes 100 sample bags with dechlorinating agent, refrigerant pack, rack and 9-L cooler	each	2568700

Optional reagents and apparatus

Description	Unit	Item no.
m-Endo broth, glass bottle	100 mL	2373542
Disposable filter funnels with membrane filters, sterile	150/pkg	2586300
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pipet, serological, 2 mL, sterile, glass	35/pkg	2093136
Pipet filler, safety bulb	each	1465100

Optional reagents and apparatus (continued)

Description	Unit	Item no.
Support base for disposable filter funnels	each	2586201
Vacuum pump, hand-operated	each	1428300

Confirmation of total coliforms (BGB and LT)

Note: *Many of the confirmation products are given in the presumptive products tables.*

Required reagents

Description	Quantity/test	Unit	Item no.
Brilliant Green Bile (BGB) Broth tubes (for total coliform confirmation)	1	15/pkg	32215
Lauryl Tryptose Broth tubes, single-strength (for total coliform confirmation)	1	15/pkg	2162315

Required apparatus

Note: *Many of the required apparatus are in the required apparatus table for confirmation of fecal coliforms (EC medium broth).*

Description	Quantity/test	Unit	Item no.
Inoculating loop, plastic disposable	1	25/pkg	2749125
Inoculating loop, nichrome wire	1	each	2112100

Confirmation of fecal coliforms (EC medium broth)

Note: *Many of the confirmation products are given in the presumptive products tables.*

Required reagents

Description	Quantity/test	Unit	Item no.
EC Medium Broth tubes (for fecal coliform confirmation)	1	15/pkg	1410415

Confirmation of *E. coli* with EC/MUG

Note: *Many of the confirmation products are given in the presumptive products tables.*

Required reagents

Description	Quantity/test	Unit	Item no.
EC Medium with MUG Broth Tubes (for E. coli confirmation)	1	15/pkg	2471515

Required apparatus

Description	Quantity/Test	Unit	Item no.
UV lamp, long-wave, portable, 4 watt	1	each	2415200
Replacement bulb for portable UV lamp	1	each	2584600
UV lamp, long-wave, 115 VAC	1	each	2184300
UV lamp, long-wave, 230 VAC	1	each	2184302
UV blocking eyewear	1	each	SM730-1033

Optional apparatus

Description	Unit	Item no.
E. coli fluorescence standard	each	2361100
Incubator, Water Bath, 120 VAC, 50/60 Hz	each	2616300
Incubator, Water Bath, 240 VAC, 50/60 Hz	each	2616302

Confirmation of *E. coli* with nutrient agar

Note: *Many of the confirmation products are given in the presumptive products tables and in the products tables for the confirmation of E. coli with EC/MUG.*

Required reagents

Description	Quantity/test	Unit	Item no.
Nutrient agar with MUG prepared plates	1	15/pkg	2812115
Nutrient agar with MUG tubes, two tests for each tube (for *E. coli* confirmation)	1	6/pkg	2437306

Membrane Filtration Bacteriological Test

Supply List (to collect)

- ☐ Vacuum pump
- ☐ Filter flask
- ☐ Vacuum hose
- ☐ Funnel (2 pieces)
  - o wrapped in brown paper.
  - o DO NOT OPEN!
- ☐ Tweezers
- ☐ Spirit lamp
- ☐ 50 mL beaker
- ☐ Alcohol

- ☐ 5 sample bottles
- ☐ 5 petri dishes
- ☐ 5 filters
- ☐ 7 dilution waters (milk bottles)
- ☐ 1 sterile water (milk bottle)

- ☐ 5 broths
- ☐ 1 control bacteria

Colilert* Test Kit

Introduction

Colilert* simultaneously detects total coliforms and *E. coli* in water. It is based on IDEXX's proprietary Defined Substrate Technology*. When total coliforms metabolize Colilert's DST* nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert's DST* nutrient-indicator, MUG, the sample also fluoresces. Colilert can simultaneously detect these bacteria at 1 cfu/100 mL within 24 hours even with as many as 2 million heterotrophic bacteria per 100 mL present.

Storage

Store at 2–30°C away from light.

Presence/Absence (P/A) Procedure

1. Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. Incubate at 35±0.5°C for 24 hours.
4. Read results according to Result Interpretation table below.

Quanti-Tray* Enumeration Procedure

1. Add contents of one pack to a 100 mL water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray* or Quanti-Tray*/2000 and seal in an IDEXX Quanti-Tray* Sealer.
4. Place the sealed tray in a 35±0.5°C incubator for 24 hours.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Less yellow than the comparator[1]	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for *E. coli*

- Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
- Colilert results are to be read after 24 hours of incubation.
- However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 28 hours total) to allow the color and/or fluorescence to intensify.
- Positives for both total coliforms and *E. coli* observed before 24 hours and negatives observed after 28 hours are also valid.
- In addition, laboratories may incubate samples for additional time (up to 28 hours total) for their convenience.

Procedural Notes

- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures. For example, samples run in other countries are incubated at 36±2°C for 24–28 hours.
- Colilert can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*[2] MPN tables should be used to find Most Probable Numbers (MPNs).
- If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.
- If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert. If this is seen, consider sample invalid and discontinue testing.
- Aseptic technique should always be followed when using Colilert. Dispose of in accordance with Good Laboratory Practices.

Quality Control Procedures

1. One of the following quality control procedures is recommended for each lot of Colilert:
   A. IDEXX-QC Coliform and *E.coli* [3]: *Escherichia coli*, *Klebsiella variicola*‡, and *Pseudomonas aeruginosa*
   B. Quanti-Cult*[4]: *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.
   C. Fill three sterile vessels with 100 mL of sterile nonbuffered oxidant-free water and inoculate with a sterile loop of ATCC[5] strains, *Escherichia coli* ATCC 25922/WDCM 00013 or ATCC 11775/WDCM 00090, *Klebsiella variicola*‡ ATCC 31488/ WDCM 00206 and *Pseudomonas aeruginosa* ATCC 10145/WDCM 00024 or ATCC 27853.
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

NOTE: IDEXX internal quality control testing is performed in accordance with ISO 11133:2014. Quality Control Certificates are available at idexx.com/water.

1. IDEXX P/A Comparator, catalog #WP104; Quanti-Tray Comparator #WQTC, or Quanti-Tray/2000 Comparator #WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
3. IDEXX-QC Coliform and *E. coli*—IDEXX Catalog #UN3373-WQC-TCEC
4. Quanti-Cult cultures—IDEXX catalog # WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org

‡. *Klebsiella pneumoniae* (ATCC 31488/WDCM 00206) has been renamed to *Klebsiella variicola*.

Bacteriological Testing

Even Number Teams		Odd Number Teams	
Tryptic Soy Broth • Positive Control (Pseudomonas)	_____	**Tryptic Soy Broth** • Negative Control (no addition)	_____

Membrane Filtration (m-Endo)
• Pre-blank (buffered water)
• River Sample
• Sample 1
• Positive TC Control
  • Klebsiella + Sterile Water
• Post-blank (buffered water)
	
	_____
	_____
	_____
	_____
	
	_____
		Membrane Filtration (m-Coliblue)
		• Pre-blank (buffered water)
		• River Sample
		• Sample 1
		• Positive EC Control
		  • E. coli + Sterile Water
		• Post-blank (buffered water)
			
			_____
			_____
			_____
			_____
			
			_____

Colilert (E. coli)
• Sample 1
• Positive EC Control
  • E. coli + Sterile Water
	
	_____
	_____
		Colilert (Klebsiella)
		• River Water
		• Negative Control
		  • Pseudomonas + Sterile Water
			
			_____
			_____

Colilert Quanti-tray
• River Water
• Negative TC & EC Control
  • Pseudomonas + Sterile Water
	
	_____
	_____
		Colilert Quanti-tray
		• Sample 1
		• Positive TC Control
		  • Klebsiella + Sterile Water
			
			_____
			_____

* “Pall” should be on bench when adding broth and filter to petri dish.
* Invert dish to incubate.
* Incubate at 35.5 +/- 0.5^{o}C for 24 hours.

Phosphorous Testing

Phosphorus

- Occurs in natural waters and wastewaters almost solely as phosphates
- Classified as
  - Orthophosphates or
  - Condensed phosphates
    - Pyrophosphates
    - Metaphosphates
    - Polyphosphates
- Occurs in solution, in particles, or in aquatic organisms

Phosphate Sources

- Some condensed phosphates added during water treatment
- Condensed phosphates may be added during laundering or other cleaning as phosphates are in many cleaners
- Orthophosphates applied as fertilizers and carried into surface waters a runoff
- Organic phosphorus formed by biological processes
  - Bodily wastes and food residues

Types of Phosphates

- Total phosphorus
  - May divided into three chemical types
- Reactive phosphorus
  - Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion
  - aka orthophosphate
  - Occurs in dissolved and suspended forms

Types of Phosphates

- Acid-hydrolyzable phosphate
  - The fraction of phosphorus in which acid hydrolysis converts dissolved and particulate condensed phosphates to dissolved orthophosphates
- Organic phosphorus
  - The fraction of phosphates that are converted to orthophosphate only by oxidation

Environmental Impact

- Phosphate will stimulate the growth of aquatic plants
- High levels of phosphates entering waterways can stimulate algae and water plants to grow wildly which chokes up the waterways and uses up large amounts of dissolved oxygen

Environmental Impact

- Eutrophication or over-fertilization of receiving water from wastewater effluents
- Digestive problems can occur from extremely high levels of phosphate

Water Stabilization

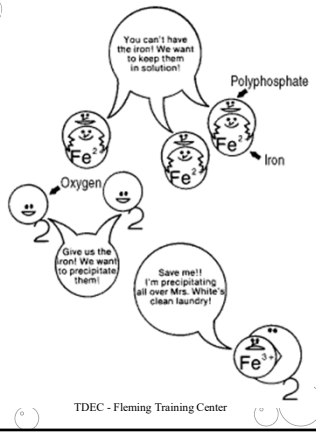
- The process for controlling corrosion and scale deposits on pipelines and plumbing fixtures
- Corrosion and scale deposits in the distribution system can be very costly for utility

Water Stabilization

- Problems range from excessive customer complaints to increased pumping costs, to replacement of mains due to leaks and breaks
- Corrosion control is also important in protecting consumers from the dangers of excess lead and copper

Polyphosphates

- Polyphoshates work as sequestering agents - tie up iron and manganese to prevent color and taste complaints
  - They also tie up calcium carbonate to prevent excess scale
  - Calcium (from alkalinity) is required as a catalyst
  - If low alkalinity, need a blend of polyphosphate and orthophosphate



Orthophosphates

- Orthophosphate coats pipe, polyphosphate sequesters
- Orthophosphates work well for lead and copper protection

Methods for Determining Phosphates

- Orthophosphate is the amount of inorganic phosphorus in a sample and is measured by direct colorimetric procedures
- Total phosphorus is the amount of all phosphorus present in the sample regardless of form and is measured by the persulfate digestion procedure followed by the colormetric analysis

Shortcut for Total Phosphorous

- If you feed a blend of ortho and poly phosphates and you know the % ortho:
  - Divide ortho mg/L by % to get approximate total concentration
  - Example: 0.32 mg/L ortho and the blend is 30% ortho
    - $0.32 \div 0.30 \approx 1.07$ mg/L total phosphate

Phosphorus, Reactive (Orthophosphate) and Total

Ascorbic Acid Method

Method 10209/10210

0.15 to 4.50 mg/L PO_4^{3-} or 0.05 to 1.50 mg/L PO_4^{3-}-P (LR)

TNTplus® 843

Scope and application: For wastewater, drinking water, boiler water, surface water and process water.

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the applicable instruments that can use TNTplus vials.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for TNTplus vials

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800	—	LZV646
DR 1900	9609900 or 9609800 (A)	—

Before starting

DR 3900, DR 3800, DR 2800: Install the light shield in Cell Compartment #2 before this test is started.
Review the safety information and the expiration date on the package.
The recommended sample pH is 2–10.
The recommended temperature for samples and reagents is 15–25 °C (59–77 °F).
The recommended temperature for reagent storage is 15–25 °C (59–77 °F).
The reagents that are used in this test contain molybdenum and are corrosive. Collect the reacted samples for proper disposal.
Use the DRB reactor with 13-mm wells for the digestion. If the reactor has 16-mm wells, insert adapter sleeves into the wells.
DR 1900: Go to All Programs>LCK or TNTplus Methods>Options to select the TNTplus number for the test. Other instruments automatically select the method from the barcode on the vial.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
Phosphorus, Reactive and Total LR TNTplus Reagent Set	1
DRB200 reactor with 13-mm wells	1

Items to collect (continued)

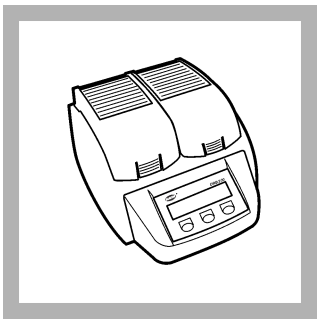
Description	Quantity
Pipet, adjustable volume, 1.0–5.0 mL	1
Pipet, adjustable volume, 0.2–1.0 mL	1
Pipet tips	1
Test tube rack	1

Refer to Consumables and replacement items on page 7 for order information.

Sample collection and storage

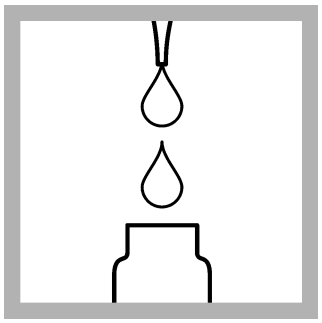
- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.
- Analyze the samples as soon as possible for best results.
- Do not use a detergent that contains phosphate to clean the sample bottles. The phosphate in the detergent will contaminate the sample.
- To preserve samples for later analysis, adjust the sample pH to 2 or less with concentrated sulfuric acid (approximately 2 mL per liter). Do not acidify samples to be analyzed only for reactive phosphorus. No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at or below 6 °C (43 °F) for a maximum of 28 days (reactive phosphorus only: 48 hours).
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5 N sodium hydroxide solution.
- Correct the test result for the dilution caused by the volume additions.

Test procedure—total phosphorus

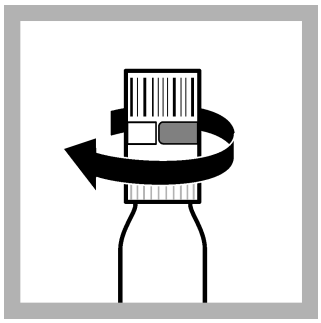


1. Set the DRB200 reactor power to on. Set the temperature to 100 °C.

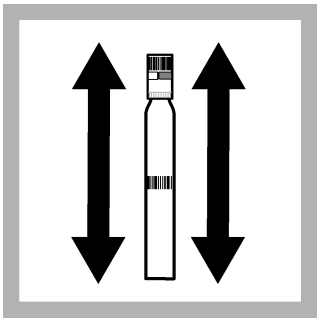
2. Carefully remove the lid from the DosiCap™ Zip cap. Remove the cap from the test vial.



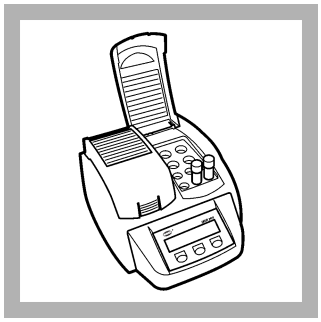
3. Use a pipet to add 2.0 mL of sample to the test vial.



4. Turn the DosiCap Zip over so that the reagent side goes on the test vial. Tighten the cap on the vial.

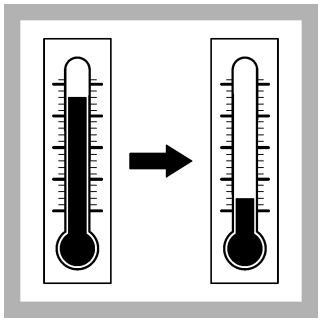


5. Shake the vial 2–3 times to dissolve the reagent in the cap.
Look through the open end of the DosiCap to make sure that the reagent has dissolved.

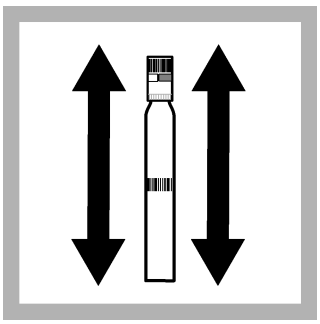


6. Insert the vial in the preheated DRB200 reactor. Close the lid.

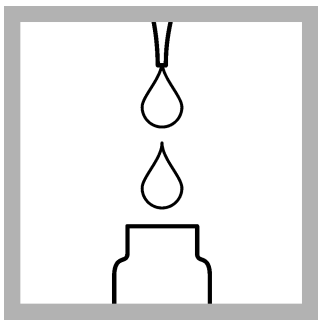
7. Keep the vial in the reactor for 1 hour.



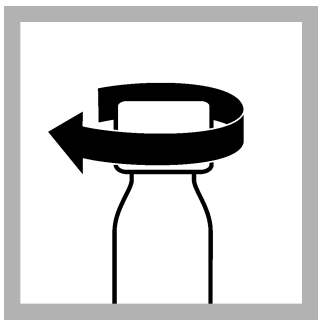
8. When the timer expires, carefully remove the vial from the reactor. Set the vial in a test tube rack. Let the temperature of the vial decrease to room temperature.



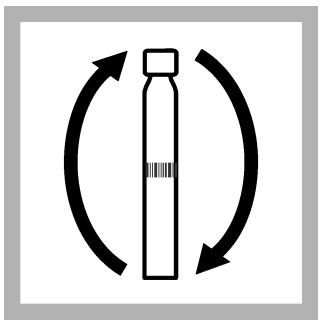
9. Shake the vial 2–3 times.



10. Use a pipet to add 0.2 mL of Solution B to the test vial. Immediately tighten the cap on the Solution B container.

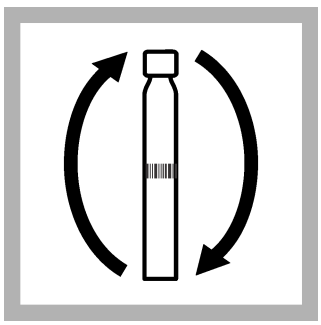


11. Put a grey DosiCap C on the vial.

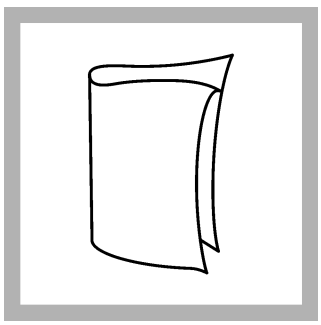


12. Tighten the cap on the vial and invert the vial 2–3 times.

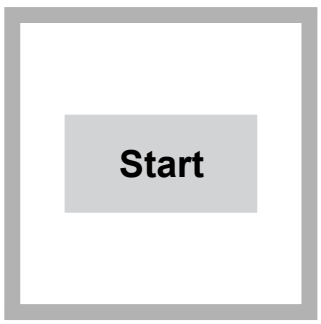
13. Start the reaction time of 10 minutes.



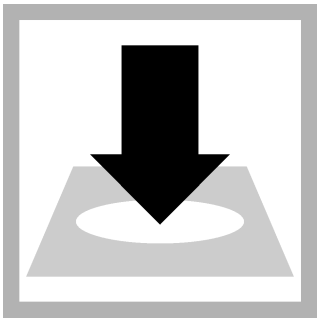
14. When the timer expires, invert the vial 2–3 times.



15. Clean the vial.

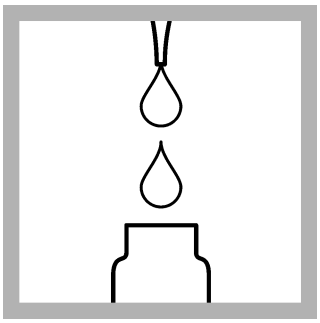


16. DR 1900 only: Select program 843. Refer to Before starting on page 1.

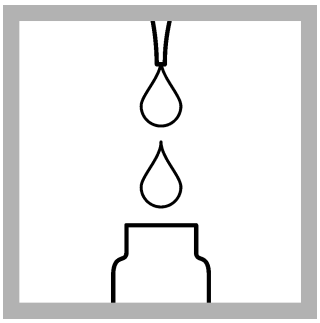


17. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in mg/L PO_4^{3-}.

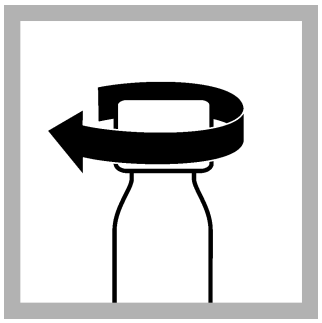
Test procedure—reactive phosphorus



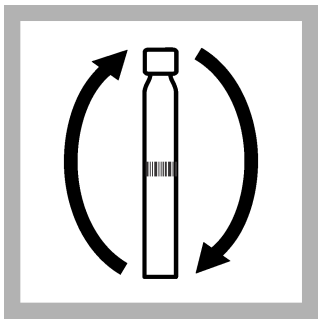
1. Use a pipet to add 2.0 mL of sample to the test vial.



2. Use a pipet to add 0.2 mL of Solution B to the test vial. Immediately tighten the cap on the Solution B container.

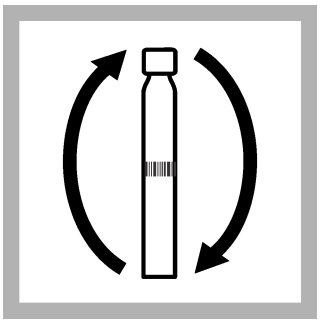


3. Put a grey DosiCap C on the vial.

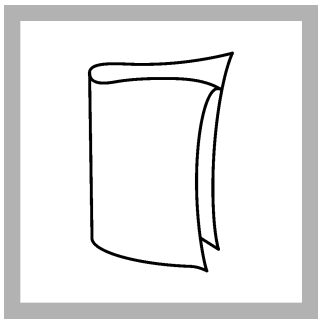


4. Tighten the cap on the vial and invert the vial 2–3 times.

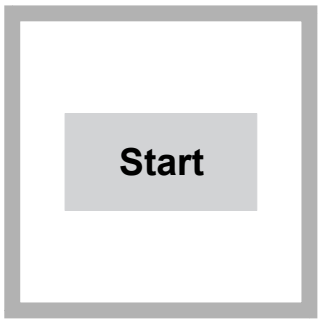
5. Start the reaction time of 10 minutes.



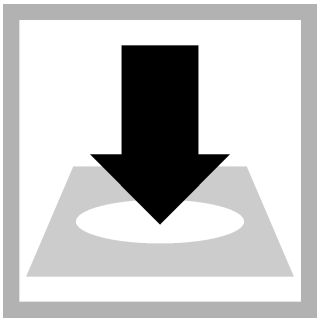
6. When the timer expires, invert the vial 2–3 times.



7. Clean the vial.



8. DR 1900 only: Select program 843. Refer to Before starting on page 1.



9. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in mg/L PO_4^{3-}.

Reagent blank correction

For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option. Measure the reagent blank value when a new lot of reagent is used.

1. Use deionized water as the sample in the test procedure to measure the reagent blank value.
2. Set the reagent blank function to on. The measured reagent blank value is shown.
3. Accept the blank value. The reagent blank value is then subtracted from all results until the reagent blank function is set to off or a different method is selected.
   Note: *As an alternative, record or enter the reagent blank value at a different time. Push the highlighted reagent blank box and use the keypad to enter the value.*

Sample blanks

Samples with color or turbidity can cause high results. Samples without color or turbidity do not require sample blanks. The digestion in the total phosphate test procedure usually removes all color and turbidity. A sample blank is not required. To adjust for color or turbidity in the reactive phosphate test procedure, use the steps that follow to find the sample blank.

1. Do the test procedure, but do not add the DosiCap C.
2. Put the cap on the vial, but do not remove the foil. Use the side of the cap that does not have the reagent.
3. Subtract the value from the final procedure step from the initial sample value to get the corrected sample concentration.
   Note: *Alternatively, samples that contain only turbidity can be filtered through a membrane filter, then analyzed.*

Interferences

Table 2 shows that the ions were individually examined to the given concentrations and do not cause interference. No cumulative effects or influences of other ions were found.

Verify the measurement results with sample dilutions or standard additions.

Table 2 Interfering substances

Interfering substance	Interference level
SO_4^{2-}	5000 mg/L
Cl^-	2000 mg/L
K^+, Na^+	1000 mg/L

Table 2 Interfering substances (continued)

Interfering substance	Interference level
NO_3^-	500 mg/L
Ca^{2+}	250 mg/L
Mg^{2+}	100 mg/L
CO_3^{2-}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, I^-, NO_2^-, Cd^{2+}, NH_4^+. Mn^{2+}, Al^{3+}, SiO_2	50 mg/L
Sn^{4+}, Hg^{2+}	5 mg/L
Ag^+, Pb^{2+}	2.5 mg/L
Cr^{3+}	1 mg/L
Cr^{6+}	0.5 mg/L

Accuracy check

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- Phosphate Standard Solution, 3-mg/L PO_4^{3-} or Wastewater Effluent Standard Solution, Mixed Parameter (contains 2-mg/L PO_4^{3-})

1. Use the test procedure to measure the concentration of the standard solution.
2. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
barcode	3.50 mg/L PO_4^{3-}	3.39–3.61 mg/L PO_4^{3-}	—

Summary of Method

Phosphates in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) are first converted to reactive orthophosphate in the total phosphorus procedure. Treatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are also converted to orthophosphates in the total phosphorus procedure by heating with acid and persulfate. The reactive phosphorus procedure measures only the reactive (ortho) phosphorus that are in the sample. The reactive or orthophosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex, which is reduced by ascorbic acid to phosphomolybdenum blue. The measurement wavelength is 880 nm (DR 1900: 714 nm).

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
Phosphorus, Reactive and Total LR TNTplus Reagent Set	1	25/pkg	TNT843

Required apparatus

Description	Quantity/test	Unit	Item no.
DRB 200 Reactor, 115 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB200-01
DRB 200 Reactor, 230 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB200-05
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Pipet, adjustable volume, 0.2–1.0 mL	1	each	BBP078
Pipet tips, for 0.2–1.0 mL pipet	2	100/pkg	BBP079
Test tube rack	1	each	1864100
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Light shield, DR 3900	1	each	LZV849

Recommended standards

Description	Unit	Item no.
Phosphate Standard Solution, 3-mg/L as PO_4^{3-}	946 mL	2059716
Wastewater Effluent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4^{3-}, COD, SO_4^{2-}, TOC	500 mL	2833249

Optional reagents and apparatus

Description	Unit	Item no.
Filter membrane, 0.45-micron, 25-mm	100/pkg	2514101
Flask, volumetric, Class A, 1000 mL glass	each	1457453
Hydrochloric Acid Solution, 6.0 N (1:1)	500 mL	88449
Reactor adapter sleeves, 16 mm to 13 mm diameter, for TNTplus vials	5/pkg	2895805
Sampling bottle with cap, low density polyethylene, 500-mL	12/pkg	2087079
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Sulfuric Acid, concentrated, ACS	500 mL	97949

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-} (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

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows adapter and light shield requirements for the instruments that use them.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for test tubes

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800, DR 2700	—	LZV646
DR 1900	9609900 (D[1])	—
DR 900	4846400	Cover supplied with the instrument

Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before this test is started.
For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option.
The test range for total phosphate is limited to 0.06 to 3.5 mg/L PO_4^{3-}. Test results that are more than 3.5 mg/L can be used to estimate dilution ratios, but should NOT be used for reporting purposes. If the test result is more than 3.5 mg/L, dilute the sample and repeat the digestion and the colorimetric test.
Clean all glassware with 6.0 N (1:1) hydrochloric acid, then fully rinse with deionized water to remove contaminants.
The reagent that is used in this test is corrosive. Use protection for eyes and skin and be prepared to flush any spills with running water.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

[1] The D adapter is not available with all instrument versions.

Items to collect

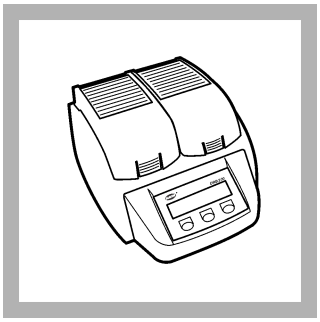
Description	Quantity
Total Phosphorus Test 'N Tube Reagent Set	1
DRB200 Reactor	1
Funnel, micro	1
Light shield or adapter (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Pipet, TenSette®, 1.0- to 10.0-mL, with pipet tips	1
Test tube rack	1
Water, deionized	varies

Refer to Consumables and replacement items on page 5 for order information.

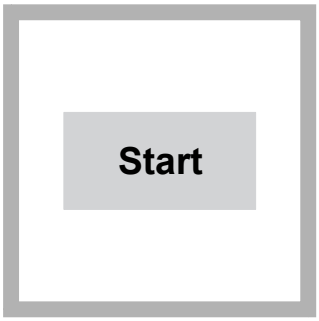
Sample collection and storage

- Collect samples in clean glass or plastic bottles that have been cleaned with 1:1 hydrochloric acid and rinsed with deionized water.
- Analyze the samples as soon as possible for best results.
- Do not use a detergent that contains phosphate to clean the sample bottles. The phosphate in the detergent will contaminate the sample.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated sulfuric acid (about 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at or below 6 °C (43 °F) for a maximum of 28 days.
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5.0 N sodium hydroxide standard solution.
- Correct the test result for the dilution caused by the volume additions.

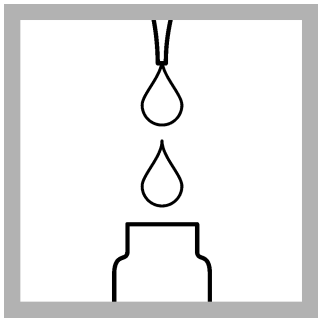
Acid persulfate digestion for Test 'N Tubes



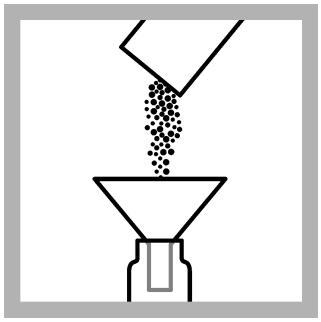
1. Start the DRB200 Reactor. Preheat to 150 °C. Refer to the DRB200 manual.



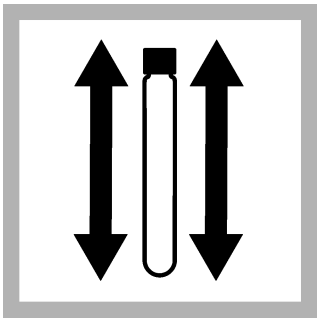
2. Start program **536 P Total/AH PV TNT**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



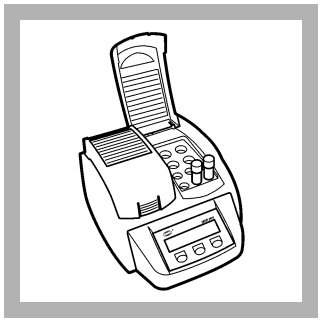
3. Add 5.0 mL of sample to the Total Phosphorus Test Vial.



4. Add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.

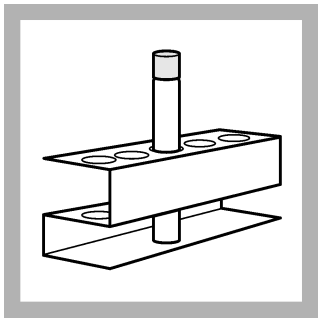


5. Put the cap on the vial. Shake to dissolve the powder.

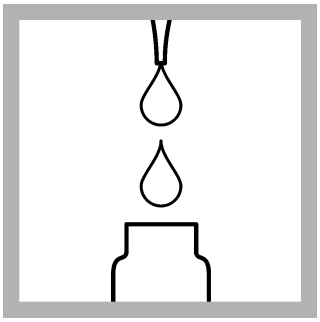


6. Insert the vial into the reactor. Close the reactor.

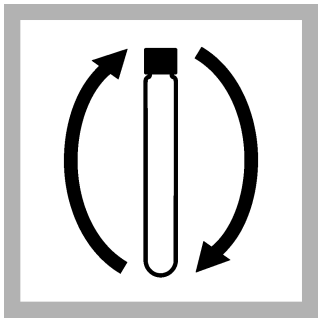
7. Start the instrument timer. A 30-minute reaction time starts.



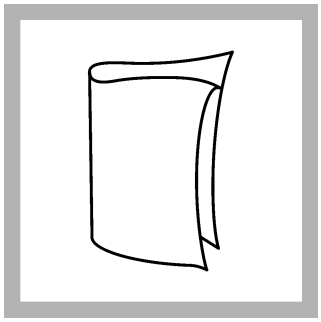
8. When the timer expires, carefully remove the vial from the reactor. Set the vial in a test tube rack. Let the vial cool to room temperature.



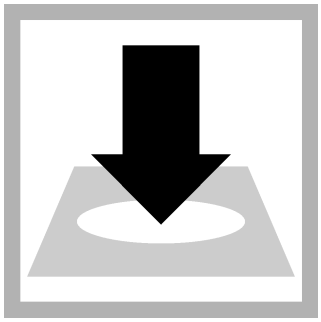
9. Add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial.



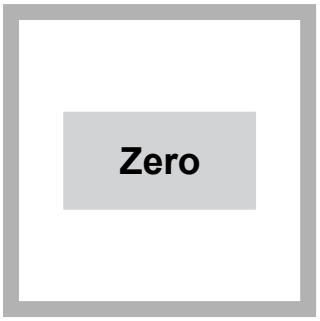
10. Put the cap on the vial. Invert to mix.



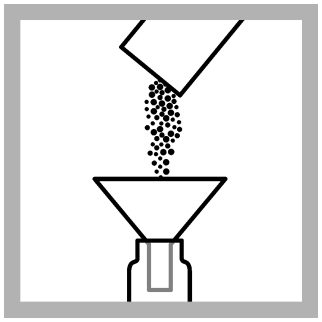
11. Clean the vial.



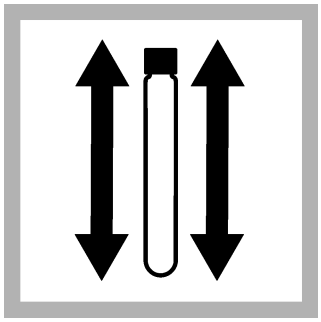
12. Insert the vial into the 16-mm cell holder.



13. Push **ZERO**. The display shows 0.00 mg/L PO_4^{3-}.



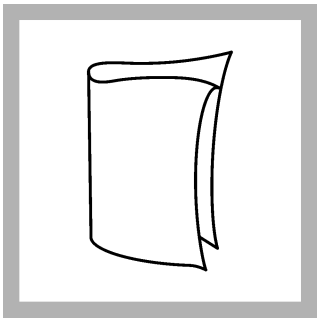
14. Add the contents of one PhosVer 3 Powder Pillow to the vial.



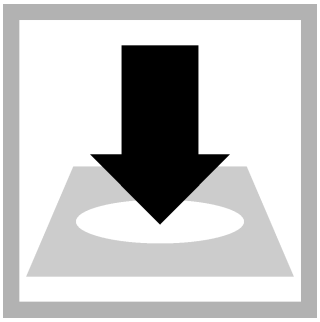
15. Put the cap on the vial. Shake to mix for 20–30 seconds. The powder will not dissolve completely.

16. Start the instrument timer. A 2-minute reaction time starts.

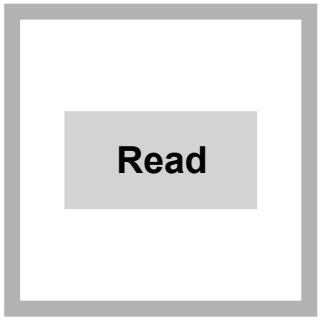
Measure the sample within 8 minutes after the timer expires.



17. Clean the vial.



18. Insert the vial into the 16-mm cell holder.



19. Push **READ**. Results show in mg/L PO_4^{3-}.

Interferences

Interfering substance	Interference level
Aluminum	More than 200 mg/L
Arsenate	Interferes at any level
Chromium	More than 100 mg/L
Copper	More than 10 mg/L
Sulfide	More than 90 mg/L
Iron	More than 100 mg/L
Nickel	More than 300 mg/L
Highly buffered samples or extreme sample pH	Can prevent the correct pH adjustment (of the sample) by the reagents. Sample pretreatment may be necessary.
Silica	More than 50 mg/L
Silicate	More than 10 mg/L
Turbidity or color	Samples with a high amount of turbidity can give inconsistent results. The acid in the reagents can dissolve some of the suspended particles and variable desorption of orthophosphate from the particles can occur.
Zinc	More than 80 mg/L

Accuracy check

Standard additions method (sample spike)

Use the standard additions method (for applicable instruments) to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- Phosphate 10-mL Ampule Standard, 50-mg/L as PO_4^{3-}
- Ampule breaker
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Mixing cylinders, 25-mL (3)

1. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
2. Go to the Standard Additions option in the instrument menu.
3. Select the values for standard concentration, sample volume and spike volumes.
4. Open the standard solution.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 25-mL portions of fresh sample. Mix well.

6. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.
7. Select **Graph** to compare the expected results to the actual results.

   Note: *If the actual results are significantly different from the expected results, make 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 the results are not within acceptable limits, the sample may contain an interference.*

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 3.0-mg/L phosphate standard solution

1. Use the test procedure to measure the concentration of the standard solution.
2. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
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. The measurement wavelength is 880 nm (DR 1900: 710 nm) for spectrophotometers or 610 nm for colorimeters.

Pollution prevention and waste management

Reacted samples contain molybdenum and must be disposed of as a hazardous waste. Dispose of reacted solutions according to local, state and federal regulations.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
Water, deionized	varies	100 mL	27242
Total Phosphorus Test 'N Tube™ Reagent Set	1	50 tests	2742645
Includes:			
PhosVer® 3 Phosphate Reagent Powder Pillow, 10 mL	1	50/pkg	2106046
Potassium Persulfate Powder Pillow	1 pillow	50/pkg	2084766

Consumables and replacement items (continued)

Description	Quantity/test	Unit	Item no.
Sodium Hydroxide, 1.54 N	varies	100 mL	2743042
Total and Acid Hydrolyzable Test Vials (not sold separately)	1	50/pkg	—

Required apparatus

Description	Quantity/test	Unit	Item no.
DRB 200 Reactor, 110 VAC option, 15 x 16-mm wells	1	each	LTV082.53.40001
DRB 200 Reactor, 220 VAC option, 15 x 16-mm wells	1	each	LTV082.52.40001
Funnel, micro, poly	1	each	2584335
Light shield, DR 3900	1	each	LZV849
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Pipet, TenSette®, 1.0–10.0 mL	1	each	1970010
Pipet tips, for TenSette® Pipet, 1.0–10.0 mL	2	250/pkg	2199725
Test tube rack	1	each	1864100

Recommended standards and apparatus

Description	Unit	Item no.
Drinking Water Standard, Mixed Parameter, Inorganic for F^-, NO_3–N, PO_4^{3-}, SO_4^{2-}	500 mL	2833049
Phosphate Standard Solution, 50-mg/L, 10-mL Voluette® Ampules	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 Effluent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4^{3-}, COD, SO_4^{2-}, TOC	500 mL	2833249
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800

Optional reagents and apparatus

Description	Unit	Item no.
Mixing cylinder, graduated, 25 mL	each	189640
Pipet, volumetric, Class A, 2 mL	each	1451536
Hydrochloric Acid Solution, 6.0 N (1:1)	500 mL	88449
Sodium Hydroxide Standard Solution, 5.0 N	1 L	245053
Sulfuric Acid, concentrated, ACS	500 mL	97949
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Bottle, sampling, with cap, low density polyethylene, 250 mL	12/pkg	2087076
Paper, pH, 0–14 pH range	100/pkg	2601300
Water, deionized	4 L	27256
Thermometer, non-mercury, –10 to +225 °C	each	2635700
Finger cots	2/pkg	1464702

Optional standards

Description	Unit	Item no.
Phosphate Standard Solution, 10-mg/L as PO_4^{3-}	946 mL	1420416
Phosphate Standard Solution, 15-mg/L as PO_4^{3-}	100 mL	1424342
Phosphate Standard Solution, 100-mg/L as PO_4	100 mL	1436832
Phosphate Standard Solution, 10-mL ampule, 500 mg/L as PO_4	16/pkg	1424210
Phosphate Standard Solution, 500-mg/L as PO_4	100 mL	1424232

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01119

USEPA[1,2] PhosVer 3® (Ascorbic Acid) Method[3] — Method 8048

0.02 to 2.50 mg/L PO_4^{3-} — Powder Pillows or AccuVac® Ampuls

Scope and application: For drinking 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] USEPA Accepted for reporting for drinking water analysis. Procedure is an acceptable version of EPA Method 365.1, approved at 40 CFR part 141 NPDWR compliance monitoring.
[3] Adapted from Standard Methods for the Examination of Water and Wastewater.

Test preparation

Instrument-specific information

Table 1 shows sample cell and orientation requirements for reagent addition tests, such as powder pillow or bulk reagent tests. Table 2 shows sample cell and adapter requirements for AccuVac Ampul tests. The tables also show all of the instruments that have the program for this test.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for reagent addition

Instrument	Sample cell orientation	Sample cell
DR 6000 DR 3800 DR 2800 DR 2700 DR 1900	The fill line is to the right.	2495402 
DR 5000 DR 3900	The fill line is toward the user.	
DR 900	The orientation mark is toward the user.	2401906 

Table 2 Instrument-specific information for AccuVac Ampuls

Instrument	Adapter	Sample cell
DR 6000 DR 5000 DR 900	—	2427606 
DR 3900	LZV846 (A)	
DR 1900	9609900 or 9609800 (C)	
DR 3800 DR 2800 DR 2700	LZV584 (C)	2122800 

Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Powder pillows

Description	Quantity
PhosVer® 3 Phosphate Reagent powder pillow, 10-mL	1
Sample cells. (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	2

Refer to Consumables and replacement items on page 6 for order information.

AccuVac Ampuls

Description	Quantity
PhosVer® 3 Phosphate Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Sample cells (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Stopper for 18-mm tubes and AccuVac Ampuls	1

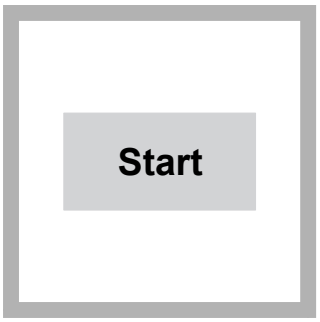
Refer to Consumables and replacement items on page 6 for order information.

Sample collection and storage

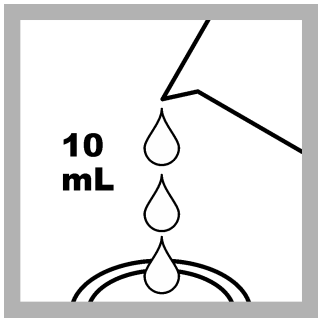
- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.

- Do not use a detergent that contains phosphate to clean the sample bottles. The phosphate in the detergent will contaminate the sample.
- Analyze the samples as soon as possible for best results.
- If immediate analysis is not possible, immediately filter and keep the samples at or below 6 °C (43 °F) for a maximum of 48 hours.
- Let the sample temperature increase to room temperature before analysis.

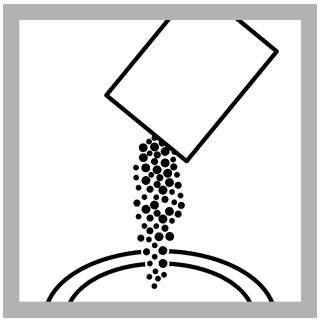
Powder pillow procedure



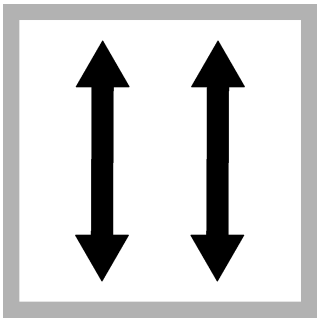
1. Start program **490 P React. PP**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



2. Prepare the sample: Fill a sample cell with 10 mL of sample.



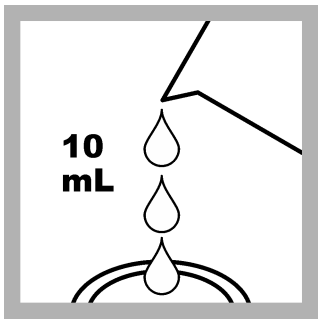
3. Add the contents of one PhosVer 3 Phosphate Reagent Powder Pillow to the cell. A blue color develops if phosphorus is in the sample.



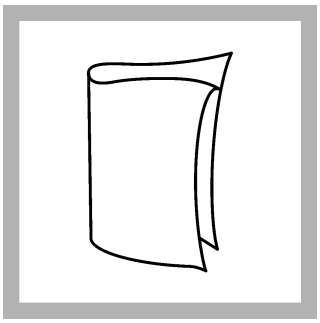
4. Immediately close the sample cell. Shake vigorously for 20–30 seconds.

5. Start the instrument timer. A 2-minute reaction time starts.

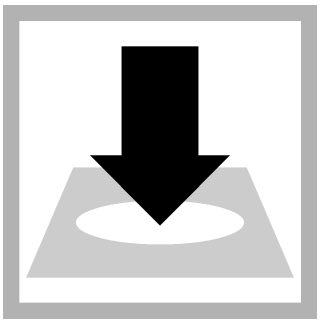
If the sample was digested using the Acid Persulfate digestion, a 10-minute reaction period is necessary.



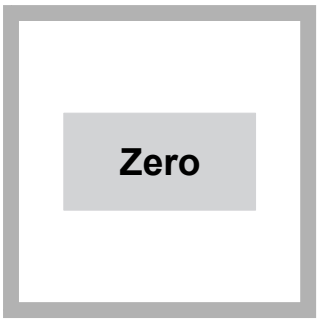
6. Prepare the blank: Fill a second sample cell with 10 mL of sample.



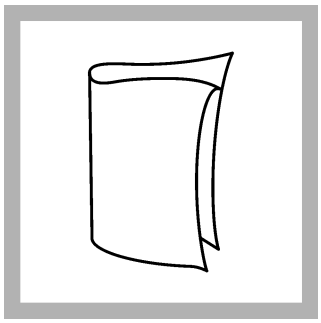
7. When the timer expires, clean the blank sample cell.



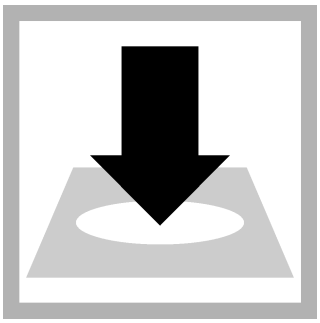
8. Insert the blank into the cell holder.



9. Push **ZERO**. The display shows 0.00 mg/L PO_4^{3-}.



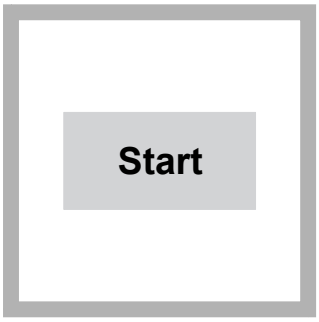
10. Clean the prepared sample cell.



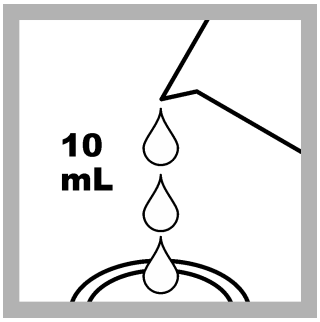
11. Insert the prepared sample into the cell holder.

12. Push **READ**. Results show in mg/L PO_4^{3-}.

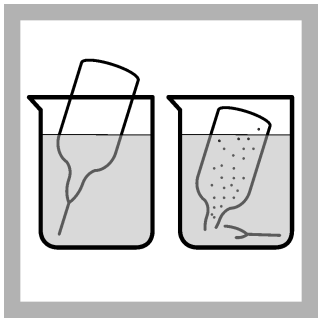
AccuVac Ampul procedure



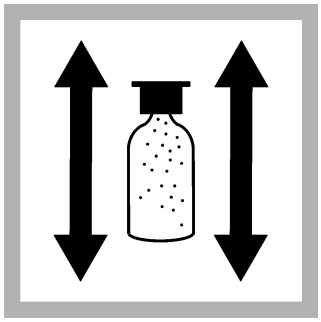
1. Start program **492 P React. PV AV**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



2. Prepare the blank: Fill the sample cell with 10 mL of sample.



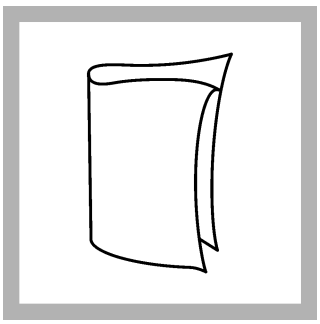
3. Prepare the sample: Collect at least 40 mL of sample in a 50-mL beaker. Fill the AccuVac Ampul with sample. Keep the tip immersed while the AccuVac Ampul fills completely.



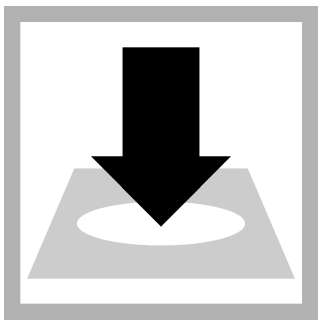
4. Close the AccuVac Ampul. Shake for approximately 30 seconds. Accuracy is not affected by undissolved powder.

5. Start the instrument timer. A 2-minute reaction time starts.

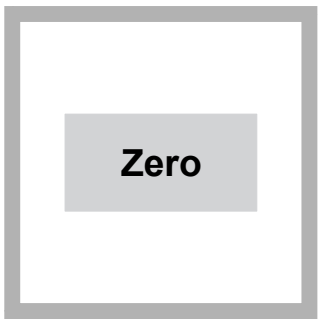
If the sample was digested using the Acid Persulfate digestion, a 10-minute reaction period is necessary.



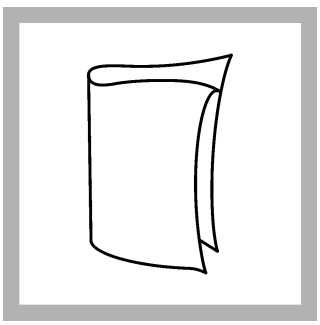
6. When the timer expires, clean the blank sample cell.



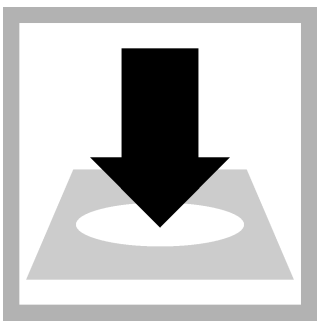
7. Insert the blank into the cell holder.



8. Push **ZERO**. The display shows 0.00 mg/L PO_4^{3-}.



9. Clean the AccuVac Ampul.



10. Insert the prepared sample AccuVac Ampul into the cell holder.

11. Push **READ**. Results show in mg/L PO_4^{3-}.

Interferences

Interfering substance	Interference level
Aluminum	More than 200 mg/L
Arsenate	Interferes at any level
Chromium	More than 100 mg/L
Copper	More than 10 mg/L
Hydrogen Sulfide	Interferes at any level
Iron	More than 100 mg/L
Nickel	More than 300 mg/L
Highly buffered samples or extreme sample pH	Can prevent the correct pH adjustment (of the sample) by the reagents. Sample pretreatment may be necessary. A pH range of 2–10 is recommended.
Silica	More than 50 mg/L
Silicate	More than 10 mg/L
Turbidity or color	Samples with a high amount of turbidity can give inconsistent results. The acid in the reagents can dissolve some of the suspended particles and variable desorption of orthophosphate from the particles can occur.
Zinc	More than 80 mg/L

Accuracy check

Standard additions method (sample spike)

Use the standard additions method (for applicable instruments) to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- Phosphate standard solution, 50 mg/L PO_4^{3-} ampule
- Ampule breaker
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Mixing cylinders, 25-mL (3)

1. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
2. Go to the Standard Additions option in the instrument menu.
3. Select the values for standard concentration, sample volume and spike volumes.
4. Open the standard solution.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 10-mL portions of fresh sample. Mix well.

   Note: *For AccuVac® Ampuls, add 0.2 mL, 0.4 mL and 0.6 mL of the standard solution to three 50-mL portions of fresh sample.*
6. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.
7. Select **Graph** to compare the expected results to the actual results.

   Note: *If the actual results are significantly different from the expected results, make 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 the results are not within acceptable limits, the sample may contain an interference.*

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 50 mg/L phosphate standard solution
- 100-mL volumetric flask, Class A
- 4-mL volumetric pipet, Class A and pipet filler safety bulb
- Deionized water

1. Prepare a 2.00-mg/L phosphate standard solution as follows:
   a. Use a pipet to add 4.00 mL of a 50-mg/L phosphate standard solution into the volumetric flask. *(Alternately, use one of the available mixed parameter standards. These standards contain 2.0 mg/L phosphate.)*
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the prepared standard solution.
3. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% Confidence Interval)	Sensitivity Concentration change per 0.010 Abs change
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, which gives an intense molybdenum blue color. The measurement wavelength is 880 nm for spectrophotometers (DR 1900: 710 nm) or 610 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
PhosVer® 3 Phosphate Reagent Powder Pillow[1], 10 mL	1	100/pkg	2106069
OR			
PhosVer® 3 Phosphate Reagent AccuVac® Ampul	1	25/pkg	2508025

Required apparatus

Description	Quantity/Test	Unit	Item no.
Beaker, 50 mL	1	each	50041H
Stoppers for 18-mm tubes and AccuVac Ampuls	2	6/pkg	173106

[1] PhosVer is a registered trademark of Hach Company.

Recommended standards

Description	Unit	Item no.
Phosphate Standard Solution, 10-mL Voluette® Ampule, 50 mg/L as PO_4	16/pkg	17110
Phosphate Standard Solution, 50 mg/L as PO_4^{3-}	500 mL	17149
Phosphate Standard Solution, 1 mg/L as PO_4^{3-}	500 mL	256949
Drinking Water Standard, Mixed Parameter, Inorganic for F^-, NO_3–N, PO_4^{3-}, SO_4^{2-}	500 mL	2833049
Wastewater Effluent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4^{3-}, COD, SO_4^{2-}, TOC	500 mL	2833249
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Item no.
AccuVac® Drainer	each	4103600
AccuVac® Ampul Snapper	each	2405200
AccuVac® Ampul vials for sample blanks	25/pkg	2677925
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
Bottle, sampling, with cap, low density polyethylene, 250 mL	12/pkg	2087076
Mixing cylinder, graduated, 50 mL	each	189641
Flask, volumetric, Class A, 100 mL, glass	each	1457442
Hydrochloric Acid Solution, 6.0 N (1:1)	500 mL	88449
Paper, pH, 0–14 pH range	100/pkg	2601300
Phosphate Treatment Powder Pillows	100/pkg	1450199
Phosphate Standard Solution, 10 mg/L as PO_4	946 mL	1420416
Phosphate Standard Solution, 15-mg/L as PO_4^{3-}	100 mL	1424342
Phosphate Standard Solution, 100-mg/L as PO_4	100 mL	1436832
Phosphate Standard Solution, 10-mL ampule, 500 mg/L as PO_4	16/pkg	1424210
Phosphate Standard Solution, 500-mg/L as PO_4	100 mL	1424232
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Pipet tips for TenSette® Pipet, 1.0–10.0 mL	50/pkg	2199796
Pipet tips for TenSette® Pipet, 1.0–10.0 mL	250/pkg	2199725
Pipet, volumetric, Class A, 4.00 mL	each	1451504

TNT843 Phosphorus, Reactive (Orthophosphate) and Total

0.05–1.50 mg/L PO_4–P or 0.15–4.50 mg/L PO_4
Low Range

TNTplus®843—
Method 10209/10210

Scope and application: For wastewater, drinking water, boiler water, surface water and process analysis.

Test preparation

Reagent storage

Storage temperature: 15–25 °C (59–77 °F)

pH/Temperature

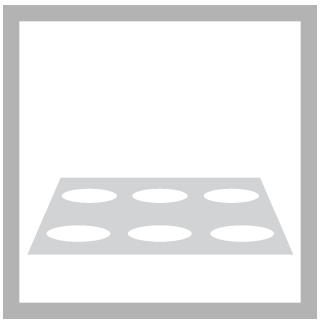
The pH of the water sample must be between pH 2–10.

The temperature of the water sample and reagents must be between 15–25 °C (59–77 °F).

Before starting

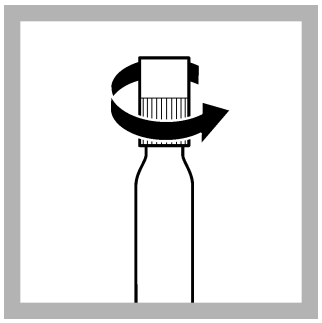
ATTENTION—Important information for the evaluation!
Without hydrolysis, only the (dissolved) orthophosphate is measured. The result of the orthophosphate measurement can be expressed as: mg/L PO_4-P (for example, process analysis), mg/L PO_4 (for example, drinking water or boiler water analysis), mg/L P_2O_5 (for example, soil analysis).
With hydrolysis, all of the phosphorus (Total-P, Ptotal) is measured. The result of the total phosphorus measurement can be expressed as: mg/L Ptot = Display mg/L PO_4-P (for example, for monitoring threshold values in wastewater), mg/L PO_4 (for example, drinking water or boiler water analysis), mg/L P_2O_5 (for example, soil analysis).
Inverting the vial after hydrolysis improves the reliability of the result.
Determination of orthophosphate: filtrate the sample before the analysis.
In case of not working at the correct recommended temperature an incorrect result may be obtained.
Review safety information and expiration date on the package.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Procedure—Phosphorus Total

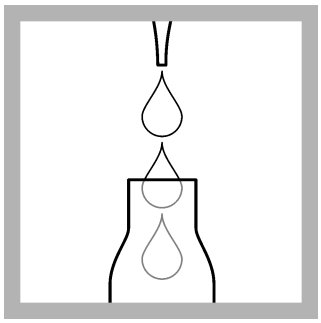


1. Preheat the reactor to **100° C (212° F)** or to **120 °C (248 °F)**.

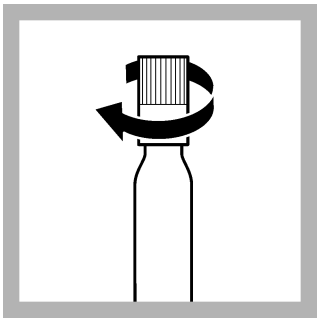
2. Carefully remove the foil from the screwed-on **DosiCap Zip A**.



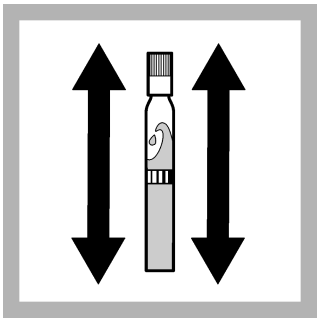
3. Unscrew the **DosiCap Zip A**.



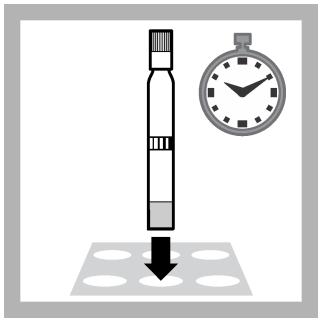
4. Carefully pipet **2.0 mL** of **sample**.



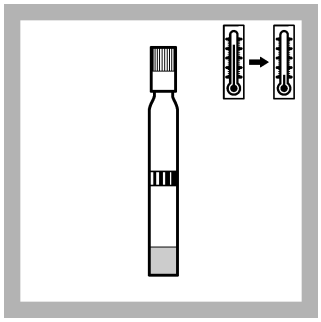
5. Immediately screw the **DosiCap Zip A** back on **tight**; **fluting at the top**.



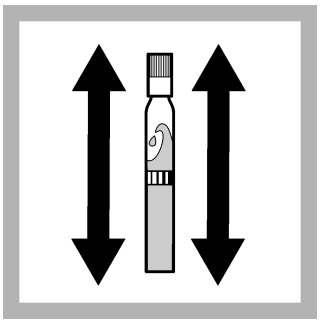
6. Shake **vigorously 2–3 x**.



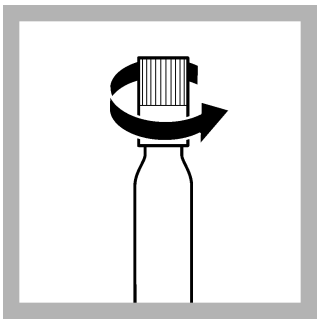
7. Heat in the reactor for **60 minutes** at **100° C (212°F)** or for **30 minutes** at **120 °C (248 °F)**.



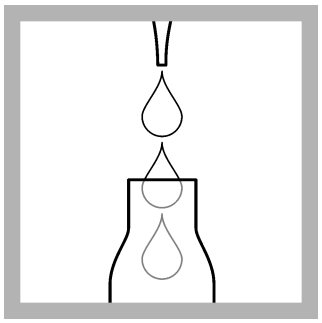
8. Allow to **cool** to room temperature.



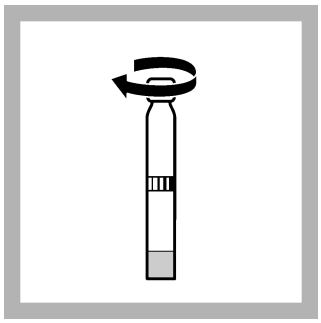
9. Shake **vigorously 2–3 x**.



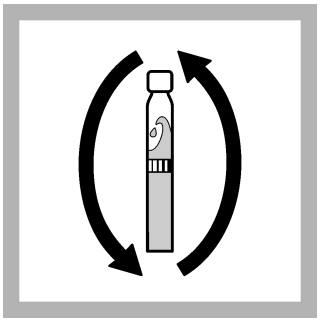
10. Unscrew the **DosiCap Zip A**.



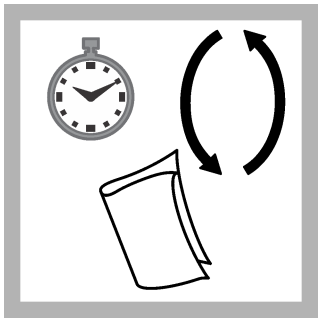
11. Pipet into the cooled vial: **0.2 mL** of **reagent B**. Close **reagent B** immediately after use.



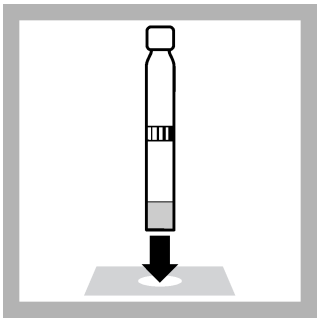
12. Screw a **grey** colored **DosiCap C** on the vial.



13. Invert a few times until the freeze-dried contents are **completely dissolved**.

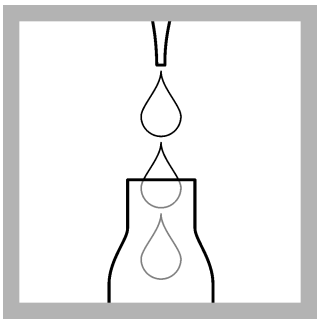


14. After **10 minutes**, invert a few more times, thoroughly clean the outside of the vial and evaluate.

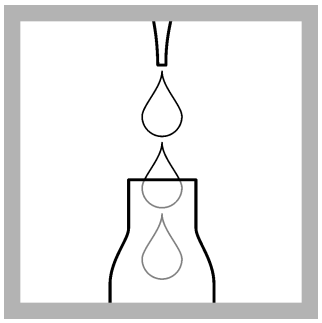


15. Insert the vial into the cell holder.
DR1900: Go to LCK/TNTplus methods.
Select the test, push **READ**.

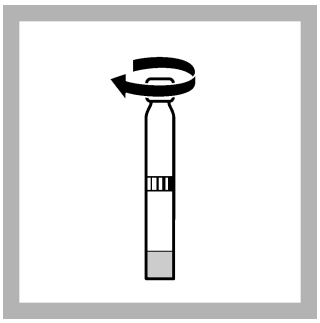
Procedure—Phosphorus Reactive (Orthophosphate)



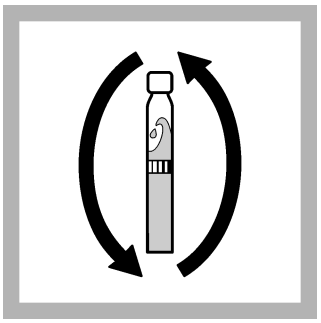
1. Carefully pipet **2.0 mL** of **sample**.



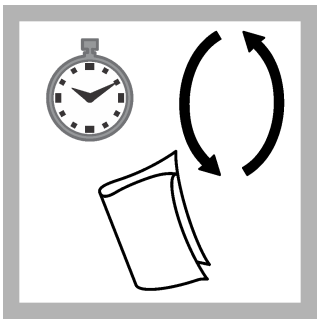
2. Pipet **0.2 mL** of **reagent B**. Close **reagent B** immediately after use.



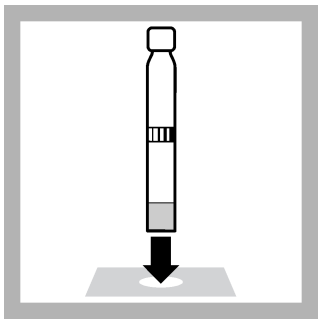
3. Screw a **grey** colored **DosiCap C** on the vial.



4. Invert a few times until the freeze-dried contents are **completely dissolved**.



5. After **10 minutes**, invert a few more times, thoroughly clean the outside of the vial and evaluate.



6. Insert the vial into the cell holder.
DR1900: Go to LCK/TNTplus methods.
Select the test, push **READ**.

Interferences

The ions listed in the table have been individually checked against the given concentrations and do not cause interference. The cumulative effects and the influence of other ions have not been determined.

The measurement results must be subjected to plausibility checks (dilute and/or spike the sample).

Interference level	Interfering substance
5000 mg/L	SO_4^{2-}
2000 mg/L	Cl^-
1000 mg/L	K^+, Na^+
500 mg/L	NO_3^-
250 mg/L	Ca^{2+}
100 mg/L	Mg^{2+}
50 mg/L	Co^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, I^-, NO_2^-, Cd^{2+}, NH_4^+, Mn^{2+}, Al^{3+}, CO_3^{2-}, SiO_2
5 mg/L	Sn^{4+}, Hg^{2+}
2.5 mg/L	Ag^+, Pb^{2+}
1 mg/L	Cr^{3+}
0.5 mg/L	Cr^{6+}

Summary of method

Phosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphormolybdate complex, which is reduced by ascorbic acid to phosphormolybdenum blue.

TNTplus®

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On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

06/2021, Edition 2

Phosphorus Lab
Bench Sheet

Total Phosphorus	**Test Results (mg/L PO_4^{3-})**	
	Tap Water	**Standard**
• TNT843		
• Test 'N Tube Vials		
• Spike (r^2)		

Reactive (Ortho) Phosphorus	**Test Results (mg/L PO_4^{3-})**	
	Tap Water	**Standard**
• TNT843		
• Powder Pillow		
• Spike (r^2)		

Lead & Copper

Water Treatment Lab

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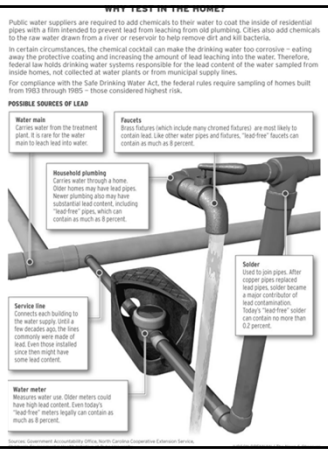
1



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2

Lead

- Primary cause of contamination in drinking water
  - Caused by corrosion of leaded materials in the water distribution system
    - Lead - based solder used to join copper pipe
    - Brass - and chrome-plated brass faucets
    - Lead service lines that connect houses to water mains

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3

Health Effects of Lead Contamination

- Children
  - Altered physical/mental development
  - Interference with growth
  - Deficits in IQ, attention span and hearing
  - Interference with heme (blood) synthesis
- Women
  - Increased blood pressure
  - Shorter gestation period
- Men
  - Increased blood pressure

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4

Health Effects of Copper Contamination

- Complications of Wilson's Disease
  - Wilson's Disease is a hereditary liver condition that restricts the body's ability to metabolize copper
- Stomach and intestinal distress
- Liver or kidney damage

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5

Treatment Techniques

- Corrosion control – Lead & Copper
- Source water treatment – Lead & Copper
- Lead service line replacement – Lead
- Public education – Lead

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6

Action Levels

- Lead = 0.015 mg/L
- Copper = 1.3 mg/L

- A system is often required to begin initiating treatment techniques when lead and/or copper levels in more than 10% of samples exceed these levels

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Water Quality Parameters

- pH
- Alkalinity
- Calcium
- Conductivity
- Water temperature
- Orthophosphate or silica
  - If an inhibitor containing these compounds is used

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8

Sample Site Selection

- All public water systems are required to sample at the customer's tap
- Specified targeting criteria have been selected to indicate probability of lead or copper contamination and to create a tiered system

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How many sampling sites?

- The number of sampling sites are determined by the size of the population served by the system

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Tier 1

- Consists of single family structures with:
  - Copper pipes with lead solder installed after 1982
  - Interior lead piping
  - Lead service lines
- May include multiple family residences if these comprise of more than 20% of the service connections to the public water supply

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Tier 2

- Consist of buildings, including multiple family residences, with:
  - Copper pipes with lead solder installed after 1982
  - Interior lead piping
  - Lead service lines

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Tier 3

- Consist of single family structures with:
  - Copper pipes with lead solder installed before 1983

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Tap Water Samples

- First draw
  - Tap water that has remained motionless in the plumbing system for at least 6 hours
  - Collected without flushing the tap
- One liter
- Collected from a cold water kitchen tap or from a bathroom sink

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Tap Water Samples

- Samples should be preserved with nitric acid (HNO_3) to a pH<2
- Plastic or glass container
- Holding time is 6 months

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Reporting

- Results of all tap samples for lead and copper
  - Location of each site
  - Criteria used for site selection
- Certification that lead and copper samples were collected properly
- Certification that residents who collected lead and copper samples were informed of correct sampling procedures

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Reporting

- Lead and copper 90th percentile concentrations
- Designation of any new sampling sites
  - Explanation of why sites have changed
- Result of all tap sampling for pH and other water quality parameters
- Results of all water quality samples collected at entry point(s) to the distribution system

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90th Percentile Concentrations

- 0400-45-1.33(1)(c)(i) – The results of all lead or copper samples taken during a monitoring period shall be placed in ascending order from the sample with the lowest concentration to the sample with the highest concentration.
- Each sampling result shall be assigned a number, ascending by single integers beginning with the number 1 for the sample with the lowest contaminant level.

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90th Percentile Concentrations

- 0400-45-1.33(1)(c)(i) cont. – The number assigned to the sample with the highest contaminant level shall be equal to the total number of samples taken.
- 0400-45-1.33(1)(c)(ii) – The number of samples taken during the monitoring period shall be multiplied by 0.9.

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90th Percentile Concentrations

- 0400-45-1.33(1)(c)(iii) – The contaminant concentration in the numbered sample yielded by the calculation in (c)3(ii) is the 90th percentile contaminant level.
- 0400-45-1.33(1)(c)(iv) – For water systems serving fewer than 100 people that collect 5 samples per monitoring period, the 90th percentile is computed by taking the average of the highest and second highest concentrations.

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90th Percentile Concentrations

- Copper Samples
  1. 0.03
  2. 0.11
  3. 0.23
  4. 0.27
  5. 0.28
  6. 0.59
  7. 0.63
  8. 0.98
  9. 1.31

0400-45-1.33(1)(c)(ii)
(0.9)(9) = 8.1

90th Percentile is less than the action level

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21

Record Keeping

- Systems must retain on its premises original records of:
  - All sampling data and analyses
  - Reports
  - Surveys
  - Letters
  - Evaluations
  - Schedules
  - State determinations
- These records must be kept for 12 years

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Lead and Copper
Review Questions

1. Name the primary cause of lead contamination in drinking water.

2. List two negative health effects of lead contamination.

3. List two negative health effects of copper contamination.

4. What are the treatment technique requirements specified in the Lead and Copper Rule?

5. What is the action level for lead and for copper?

6. For community water systems, Tier 1 sampling sites consist of single family structures with:
   a _____
   b _____
   c _____

7. All tap water samples must be _____ in volume and must have stood motionless in the plumbing system of the sampling site for at least _____ hours.

8. True or False. The number of sampling sites is determined by the size of the population served by the system.

9. The Lead and Copper Rule specifies that systems must monitor the following water quality parameters:
   a _____
   b _____
   c _____
   d _____
   e _____
   f _____
   g _____

10. How many years must the system keep records required by the Lead and Copper Rule?

11. How do you preserve a sample for Lead and Copper and what is the holding time?

Answers

1. Corrosion of lead-containing materials in distribution and plumbing systems.
2. Negative health effects of lead contamination include:
   a Increased blood pressure
   b Shorter gestational period for pregnant women
   c Brain, red blood cell and kidney damage
   d Deficits in IQ, attention span and hearing in children
   e Interference with growth in children
   f Interference with heme (blood) synthesis in children
   g Slowed mental and physical development in children
3. Negative health effects of copper contamination include:
   a Stomach and intestinal distress
   b Complications of Wilson's disease
   c Liver and kidney damage
   d Anemia
4. The treatment technique requirements specified in the Lead and Copper Rule are:
   a For lead: corrosion control treatment, source water treatment, lead service line replacement, public education
   b For copper: corrosion control treatment and source water treatment
5. The action level for lead is exceeded if more than 10% of samples have lead concentrations greater than 0.015 mg/L (15 ppb). The action level for copper is exceeded if more than 10% of samples have copper concentrations greater than 1.3 mg/L.
6. For CWSs, Tier 1 sampling sites consist of single family structures with:
   a Copper pipes with lead solder installed after 1982
   b Interior lead piping
   c Lead service lines
7. All tap water samples must be **1L** in volume and must have stood motionless in the plumbing system of the sampling site for at **6** hours.
8. True
9. The Lead and Copper Rule specifies that systems must monitor the following water quality parameters:
   a pH
   b Alkalinity
   c Orthophosphate
   d Silica
   e Conductivity
   f Calcium
   g Water temperature
10. at least 12 years
11. Preserve with HNO_3 (nitric acid) to a pH<2 in a plastic or glass container with a holding time of 6 months.

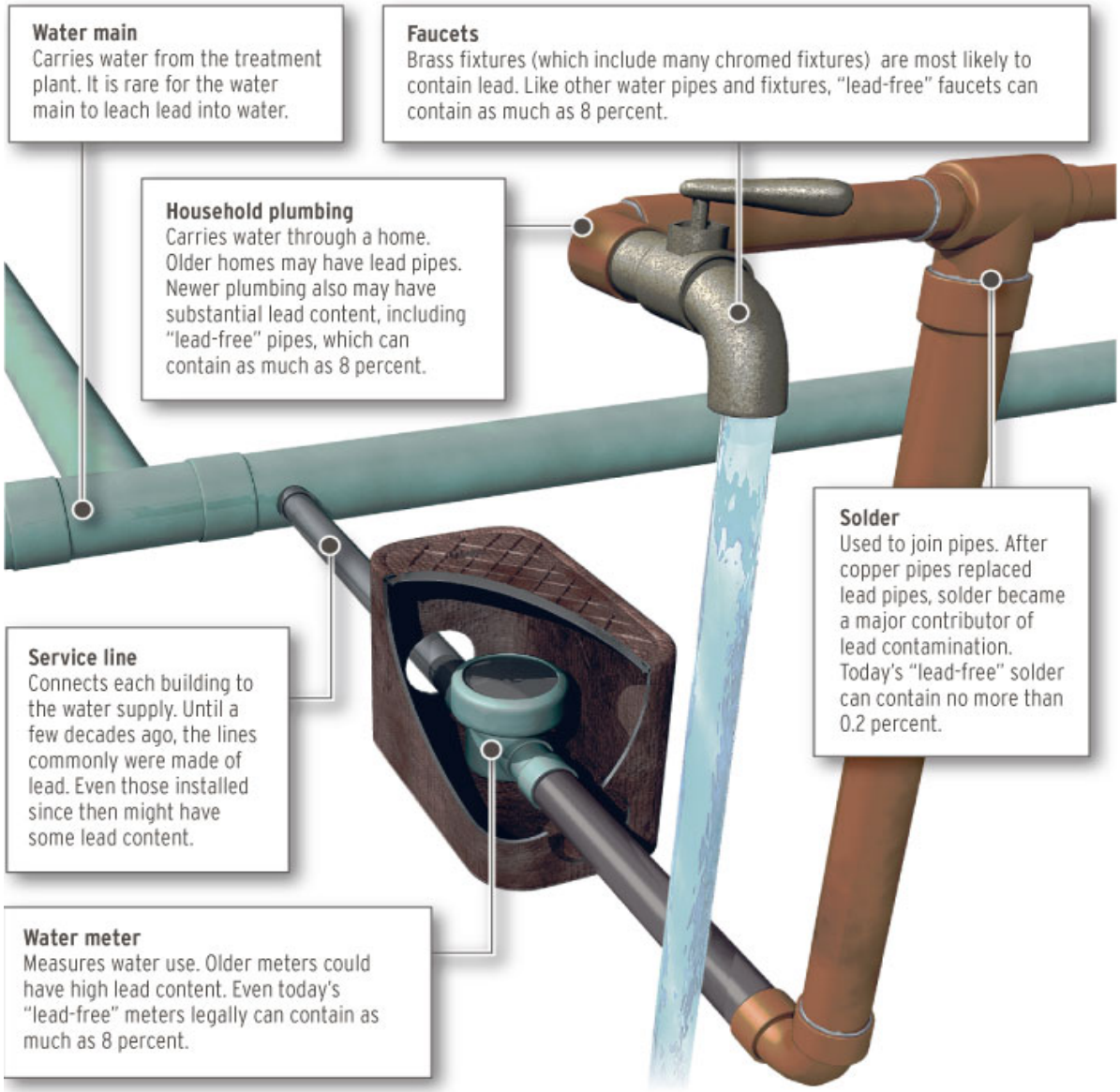
WHY TEST IN THE HOME?

Public water suppliers are required to add chemicals to their water to coat the inside of residential pipes with a film intended to prevent lead from leaching from old plumbing. Cities also add chemicals to the raw water drawn from a river or reservoir to help remove dirt and kill bacteria.

In certain circumstances, the chemical cocktail can make the drinking water too corrosive – eating away the protective coating and increasing the amount of lead leaching into the water. Therefore, federal law holds drinking water systems responsible for the lead content of the water sampled from inside homes, not collected at water plants or from municipal supply lines.

For compliance with the Safe Drinking Water Act, the federal rules require sampling of homes built from 1983 through 1985 – those considered highest risk.

POSSIBLE SOURCES OF LEAD



Sources: Government Accountability Office, North Carolina Cooperative Extension Service, Children's Environmental Health Initiative at Duke University

JUDSON DRENNAN / The News & Observer

Copper

DOC316.53.01255

Bathocuproine Method[1]

Method 10238

0.1 to 8.0 mg/L Cu

TNTplus™ 860

Scope and application: For water, wastewater and process water. Digestion can be necessary to determine total copper.

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

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the applicable instruments that can use TNTplus vials.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for TNTplus vials

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800	—	LZV646
DR 1900	9609900 or 9609800 (A)	—

Before starting

DR 3900, DR 3800, DR 2800: Install the light shield in Cell Compartment #2 before this test is started.
Review the safety information and the expiration date on the package.
The recommended sample pH is 2.5–11.
The recommended temperature for samples and reagents is 15–25 °C (59–77 °F).
The recommended temperature for reagent storage is 15–25 °C (59–77 °F).
To make sure that all forms of the metal are measured, digest the sample with heat and acid. Use the Metals Prep Set TNTplus 890 to digest the sample.
DR 1900: Go to All Programs>LCK or TNTplus Methods>Options to select the TNTplus number for the test. Other instruments automatically select the method from the barcode on the vial.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

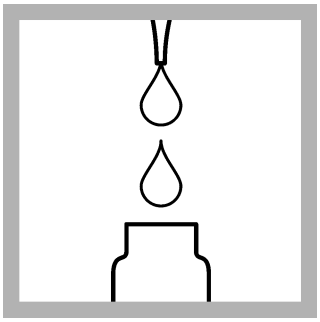
Description	Quantity
Copper TNTplus reagent set	1
Pipet, adjustable volume, 1.0–5.0 mL	1
Pipet tips, for 1.0–5.0 mL pipet	1

Refer to Consumables and replacement items on page 3 for order information.

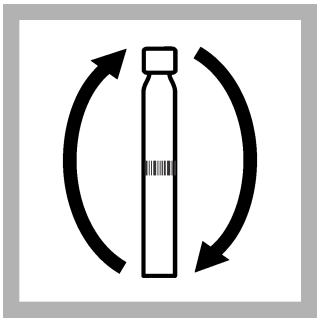
Sample collection and storage

- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated nitric acid (approximately 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- To determine dissolved copper, filter the sample before the acid addition.
- Keep the preserved samples at room temperature for a maximum of 6 months.
- Before analysis, adjust the pH to 4–6 with 8 N potassium hydroxide. Do not exceed pH 6 to prevent a copper precipitate.
- Correct the test result for the dilution caused by the volume additions.

Test procedure

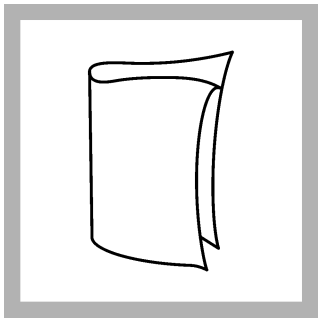


1. Use a pipet to add 2.0 mL of sample to the test vial.

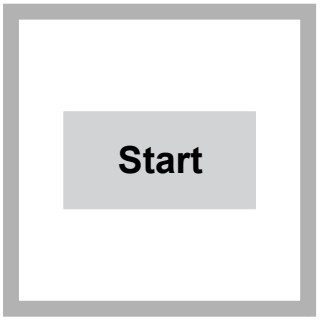


2. Tighten the cap on the vial and invert until completely mixed.

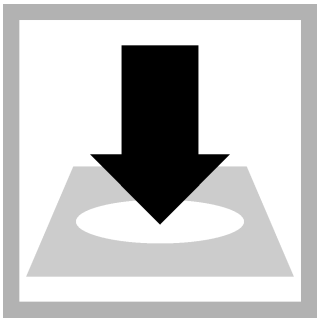
3. Start the reaction time of 3 minutes.



4. When the timer expires, clean the vial.



5. DR 1900 only: Select program 860. Refer to Before starting on page 1.



6. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in mg/L Cu.

Interferences

Table 2 shows that the ions and levels were individually examined to the given concentrations and do not cause interference. No cumulative effects or influences of other ions were found.

Higher amounts of iron and chromium can cause high-bias results. Undissolved copper contained in complexes is only found after digestion.

Table 2 Interfering substances

Interfering substance	Interference level
Sn^{2+}, Hg^{2+}	5 mg/L
Fe^{2+}, Fe^{3+}	15 mg/L
Cr^{3+}, Cr^{6+}	25 mg/L
Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}	50 mg/L
Mg^{2+}, K^{+}, Na^{+}, NH_4^{+}, Ca^{2+}, PO_4^{3-}, CO_3^{2-}, NO_2^{-}	500 mg/L
Cl^{-}), NO_3^{-}, SO_4^{2-}	1000 mg/L

Accuracy check

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 100-mg/L Copper Standard Solution
- 100-mL volumetric flask, Class A
- 4-mL volumetric pipet, Class A and pipet filler safety bulb
- Deionized water

1. Prepare a 4.0-mg/L copper standard solution as follows:
   a. Use a pipet to add 4.0 mL of a 100-mg/L copper standard solution into the volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the prepared standard solution.
3. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are slight variations in the reagents or instruments.*

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
barcode	4.0 mg/L Cu	3.9–4.1 mg/L Cu	0.04 mg/L Cu

Summary of Method

Copper (I) ions form an orange complex with the disodium salt of bathocuproine disulphonic acid. Copper (II) ions in the water sample are reduced to copper (I) ions by ascorbic acid before the complex is formed. The measurement wavelength is 478 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
Copper TNTplus reagent set	1	25/pkg	TNT860

Required apparatus

Description	Quantity/test	Unit	Item no.
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Light shield, DR 3900	1	each	LZV849

Recommended standards

Description	Unit	Item no.
Copper Standard Solution, 100-mg/L as Cu	100 mL	12842

Optional reagents and apparatus

Description	Unit	Item no.
DRB 200 Reactor, 115 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	each	DRB20001
Flask, volumetric, Class A, 100-mL glass	each	1457442
Metals Prep Set TNTplus	50/pkg	TNT890
Nitric Acid, concentrated	500 mL	15249
Potassium Hydroxide Solution, 8 N	100 mL MDB	28232H
Sampling bottle with cap, low density polyethylene, 500-mL	12/pkg	2087079
Water, deionized	4 L	27256

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Lead

DOC316.53.01056

PAR Method **Method 10216**

0.1 to 2.0 mg/L Pb **TNTplus™ 850**

Scope and application: For wastewater and process control.

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the applicable instruments that can use TNTplus vials.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for TNTplus vials

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800	—	LZV646
DR 1900	9609900 or 9609800 (A)	—

Before starting

DR 3900, DR 3800, DR 2800: Install the light shield in Cell Compartment #2 before this test is started.
Review the safety information and the expiration date on the package.
The recommended sample pH is 3–9.
The recommended temperature for samples and reagents is 15–25 °C (59–77 °F).
The recommended temperature for reagent storage is 2–8 °C (35–46 °F).
Samples that do not contain complexing agents and have a pH between 3 and 6 can be analyzed directly. Samples that have a pH between 6 and 9 must be digested with the Metals Prep Set TNTplus 890 to bring undissolved lead hydroxide or complex lead compounds into solution.
DR 1900: Go to All Programs>LCK or TNTplus Methods>Options to select the TNTplus number for the test. Other instruments automatically select the method from the barcode on the vial.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
Lead TNTplus Reagent Set	1
Pipet, adjustable volume, 0.2–1.0 mL	1
Pipet, adjustable volume, 1.0–5.0 mL	1
Pipet tips	1

Items to collect (continued)

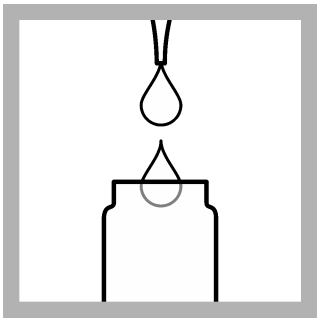
Description	Quantity
Pipet, volumetric, Class A, 10.0 mL	1
Pipet filler, safety bulb	1

Refer to Consumables and replacement items on page 4 for order information.

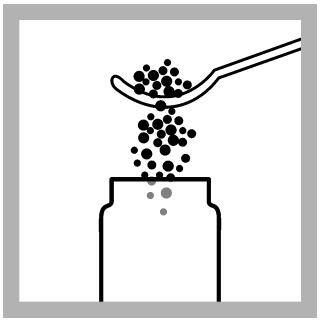
Sample collection and storage

- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated nitric acid (approximately 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at room temperature for a maximum of 6 months.
- Before analysis, adjust the pH to 3–6 with 5 N sodium hydroxide solution.
- Correct the test result for the dilution caused by the volume additions.

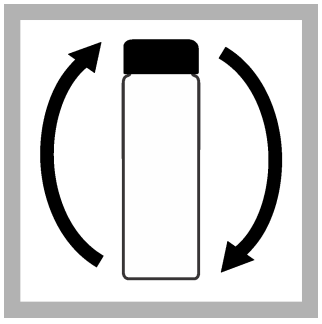
Test procedure



1. Use a pipet to add 10 mL of sample to a 20-mm reaction tube.

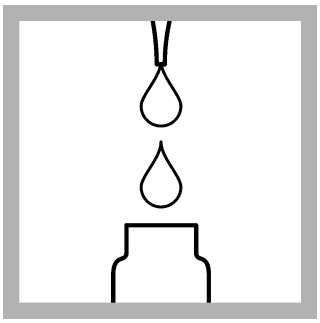


2. Add one level spoonful of Reagent A to the reaction tube.

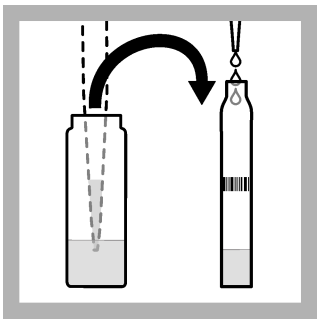


3. Tighten the cap on the reaction tube and invert the vial 2–3 times.

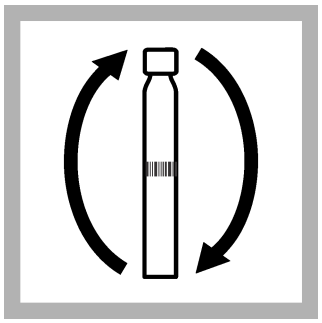
4. Start the reaction time of 2 minutes.



5. Use a pipet to add 1.5 mL of Solution B to the test vial.

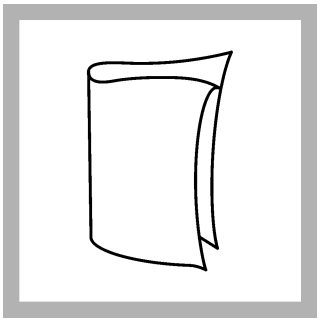


6. Use a pipet to add 4.0 mL of the treated sample from the 20-mm reaction tube to the vial.

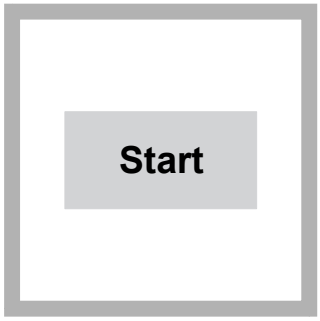


7. Tighten the cap on the vial and invert the vial 2–3 times.

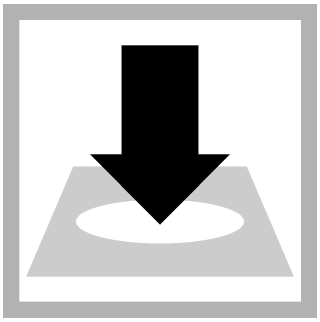
8. Start the reaction time of 2 minutes.



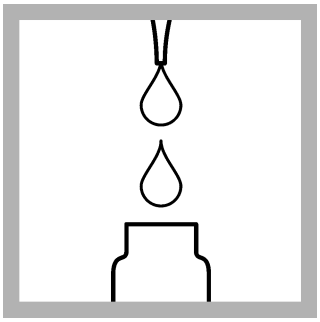
9. When the timer expires, clean the vial.



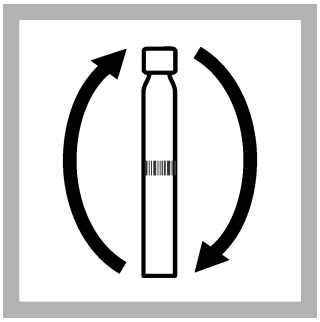
10. DR 1900 only: Select program 850. Refer to Before starting on page 1.



11. Insert the vial into the cell holder. DR 1900 only: Push **READ1**. The instrument zero is set.

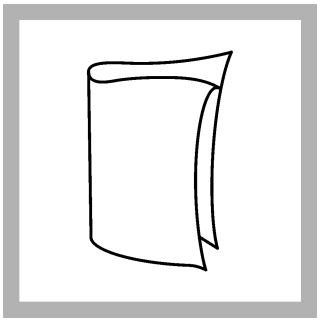


12. Use a pipet to add 0.3 mL of Solution C to the test vial.

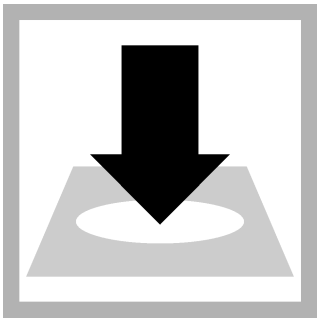


13. Tighten the cap on the vial and invert the vial 2–3 times.

14. Start the reaction time of 1 minute.



15. When the timer expires, clean the vial.



16. Insert the vial into the cell holder. DR 1900 only: Push **READ2**.
Results show in mg/L Pb.

Reagent blank correction

For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option. Measure the reagent blank value when a new lot of reagent is used.

1. Use deionized water as the sample in the test procedure to measure the reagent blank value.
2. Set the reagent blank function to on. The measured reagent blank value is shown.
3. Accept the blank value. The reagent blank value is then subtracted from all results until the reagent blank function is set to off or a different method is selected.
   Note: *As an alternative, record or enter the reagent blank value at a different time. Push the highlighted reagent blank box and use the keypad to enter the value.*

Interferences

Table 2 shows that the ions listed have been individually examined to the given concentrations and do not cause interference. No cumulative effects or influences of other ions were found.

Verify the measurement results with sample dilutions or standard additions.

Table 2 Interfering substances

Interfering substance	Interference level
K^+, Na^+, Ca^{2+}, Mg^{2+}, NO_3^-, Cl^-, PO_4^{3-}, CO_3^{2-}, SO_4^{2-}	500 mg/L
F^-, NH_4^+, Sr^{2+}	50 mg/L
Ag^+, Cd^{2+}, Cr^{6+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}	25 mg/L

Table 2 Interfering substances (continued)

Interfering substance	Interference level
Cr^{3+}, Al^{3+}, Fe^{2+}, Fe^{3+}	10 mg/L
Mn^{2+}, Hg^{2+}	5 mg/L
Sn^{2+}	0.5 mg/L

Accuracy check

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 100-mg/L Lead Standard Solution
- 100-mL volumetric flask, Class A
- 1.0-mL volumetric pipet, Class A and pipet filler safety bulb
- Deionized water

1. Prepare a 1.0-mg/L lead standard solution as follows:
   a. Use a pipet to add 1.0 mL of a 100-mg/L lead standard solution into the volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the prepared standard solution.
3. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

Summary of Method

Lead (II) ions react at pH 9 with 4-(2-pyridylazo)-resorcinol (PAR) to form a red complex. The measurement wavelength is 520 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
Lead TNTplus Reagent Set	1	25/pkg	TNT850

Required apparatus

Description	Quantity/test	Unit	Item no.
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Light shield, DR 3900	1	each	LZV849

Recommended standards

Description	Unit	Item no.
Lead Standard Solution, 100-mg/L Pb	100 mL	1261742

Optional reagents and apparatus

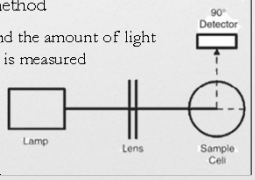
Description	Unit	Item no.
DRB 200 Reactor, 115 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	each	DRB20001
DRB 200 Reactor, 230 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	each	DRB20005
Metals Prep Set TNTplus	50/pkg	TNT890
Nitric Acid, concentrated	500 mL	15249
Sampling bottle with cap, low density polyethylene, 500-mL	12/pkg	2087079
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Water, deionized	4 L	27256

Turbidity

AWWA WSO: Water Quality
Chapter 5
CSUS: Water Treatment Plant Operation, Vol 1
Chapter 9

Turbidity

- Expression of the physical cloudiness of water
- Caused by the presence of suspended matter such as silt, finely divided organic and inorganic matter, and microscopic organisms such as algae
- Measured using nephelometric method
  - Passes light through a sample and the amount of light deflected, usually at a 90° angle, is measured



Turbidity

- Measured in turbidity units (TUs) of various kinds
  - Formazin Turbidity Unit (FTU)
    - Used when formazin is the standard
  - Nephelometric Turbidity Units (NTU)
    - Most common and interchangeable with FTU
- SDWA requires turbidity to be measured every 4 hours or continuously
- Most critical tool in efficient plant operation

Turbidity

- Turbidity vs Suspended solids
  - Turbidity expresses how much light is scattered by sample (NTU)
  - Suspended solids measurements express weight of suspended materials in sample (mg/L)
- Interferences
  - Color
  - High Turbidity
  - Gas Bubbles
  - Scratched Sample Cells
  - Condensation
  - Poor Calibration
  - Stray Light

Significance

- > 5 NTU noticeable to customers
- Increased turbidity decreases disinfection efficiency
  - Can shelter pathogens allowing viable microorganisms to reach customers
  - Can create disinfection by-products (DBPs)
- Monitor turbidity for finished water quality compliance with state and federal standards
- In-plant analysis can be used to evaluate treatment operations

Significance

- Changes in raw water turbidity usually require a change in coagulant dose
- Measurements taken after settling and before filtration reflect performance of coagulation, flocculation, and sedimentation processes
  - Increase in turbidity here indicates the coagulant application should be changed and/or operational changes made
  - Turbidity should be < 10 ntu to minimize load on filter

Rules of Public Water Systems

- 0400-45-01-.08(2)(a) Turbidity measurements must be performed on representative samples of the system's filtered water **every four hours**
- 0400-45-01-.08(4)(b) Surface water systems and ground water systems under the direct influence of surface water - Turbidity measurements must be reported within **10 days** after the end of each month the system serves water to the public.
- 0400-45-01-.17(40) Benchtop and continuous turbidimeters used to determine compliance with limits set forth in this rule chapter must be calibrated at least every three months with primary standards and documented. Documentation shall be maintained for a period not less than **five years**.

Rules of Public Water Systems

- 0400-45-01-.17(41)(a) Verification of benchtop turbidimeters must be performed daily and documented. Verifications must include a sample in the expected working range of the instrument or as close to the working range as possible. Documentation must include assigned reference material value after calibration, recorded daily reading for all reference standards, instrument identification, and date.

Rules of Public Water Systems

- 0400-45-01-.31(4)(c)

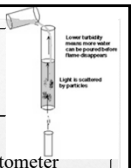
  1. For systems using conventional filtration or direct filtration, the turbidity level of representative samples of a system's filtered water must be less than or equal to 0.3 NTU in at least 95 percent of the measurements taken each month…

  2. The turbidity level of representative samples of a system's filtered water must at no time exceed 1 NTU…

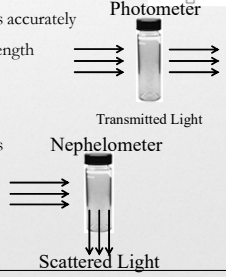
Turbidity

- Procedure
  - Follow manufacturer's instructions to calibrate turbidimeter
    - If already calibrated, verify calibration
  - Measurements
    - Clean sample tube inside and out
    - Measure sample as soon as possible
      - Shaking sample will not re-create lost turbidity
    - Agitate sample enough to assure particle suspension, but not so much to introduce bubbles
    - Do not dilute samples unless absolutely necessary

Measuring Turbidity

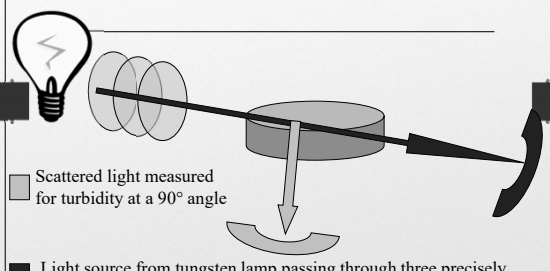


- Jackson Candle
  - Could not measure low turbidities accurately
  - Candle wick burned at a certain length
- Photometers
  - Measured "transmitted light"
  - Could not measure low turbidities
- Nephelometers
  - Detector is 90° from light source



11

Turbidimeters



Scattered light measured for turbidity at a 90° angle

Light source from tungsten lamp passing through three precisely aligned lenses, the light is focused in a narrow, collimated beam

Instruments

HACH 2100-N Laboratory Turbidimeter Benchtop (obselete)

Portable Turbidimeter

Measuring Notes

- Always cap the sample cell to prevent spillage into instrument
- Close the sample compartment lid during measurement
- Do not leave sample cell in the cell compartment for extended periods of time
- Leave the instrument on 24 hours a day if instrument is used regularly

Measuring Notes

- Always use clean, scratch free sample cells and caps
- Always use silicone oil
  - Do **NOT** use silicone oil for **new** TU series
- Measuring samples immediately to prevent changes in sample characteristics
- Remove air bubbles in sample cells
- Discard sample cells with scratches

Measuring Turbidity Sources of Error

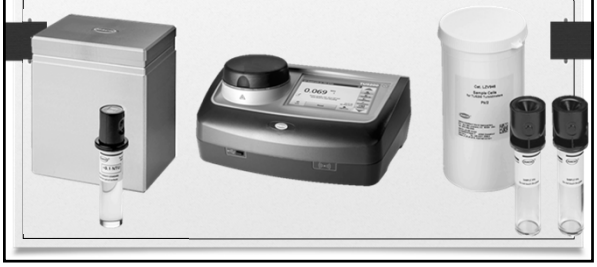
- Contaminated cells
  - Wash cells with detergent and HCL acid
  - Use ultrasonic bath
  - Rinse with filtered DI water very regularly (5-15 times)
- Scratched cells
  - Silicone oil has some refractive properties as sample cell
  - Do not use silicone oil with new TU5 series turbs

Measuring Turbidity Sources of Error

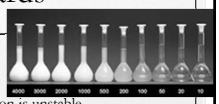
- Gas bubbles
  - Sample cell probably dirty
- Stray light
  - Keep sample compartment and optics clean
- Improper calibration

TU5 Series

- https://youtu.be/ZAGkXgDaHYk?list=PLL5AjVHeGuHm4_cih1-H_CojnAmJve92W



Turbidity Standards



- Formazin
  - Purchased as 4000 NTU
    - Diluted immediately prior to use as dilution is unstable
  - EPA approved
  - Can be made by operators when following Standard Methods
- StablCal
  - Purchased at required NTU value
  - Liquid matrix to make dilution is not water so allows longer stability
    - Stable for 1 year
  - EPA approved

Turbidity Standards

- Gelex Secondary Standards
  - Approximate turbidity range
  - No shelf life or expiration date
  - Should read within 10% of assigned value after turbidimeter calibration
- Primary standards are used for calibration (every 90 days)
  - Formazin
  - StablCal
- Secondary Standards are used for verification (daily)
  - Gelex
  - Gel standards should never be used for calibration

Calibration

- Calibrate regularly with primary standards at values recommended by manufacturer
  - Hach recommends standards no less than 20 NTU
  - Small amounts of error (0.23) in a 20 NTU standard prepared by operator will not affect accuracy
  - However, 0.23 error in a 0.5 NTU will greatly affect accuracy of low level measurements

2130 TURBIDITY*

2130 A. Introduction

1. Sources and Significance

Clarity of water is important in producing products destined for human consumption and in many manufacturing operations. Beverage producers, food processors, and potable water treatment plants drawing from a surface water source commonly rely on fluid-particle separation processes such as sedimentation and filtration to increase clarity and ensure an acceptable product. The clarity of a natural body of water is an important determinant of its condition and productivity.

Turbidity in water is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample. Correlation of turbidity with the weight or particle number concentration of suspended matter is difficult because the size, shape, and refractive index of the particles affect the light-scattering properties of the suspension. When present in significant concentrations, particles consisting of light-absorbing materials such as activated carbon cause a negative interference. In low concentrations these particles tend to have a positive influence because they contribute to turbidity. The presence of dissolved, color-causing substances that absorb light may cause a negative interference. Some commercial instruments may have the capability of either correcting for a slight color interference or optically blanking out the color effect.

2. Selection of Method

Historically, the standard method for determination of turbidity has been based on the Jackson candle turbidimeter; however, the lowest turbidity value that can be measured directly on this device is 25 Jackson Turbidity Units (JTU). Because turbidities of water treated by conventional fluid-particle separation processes usually fall within the range of 0 to 1 unit, indirect secondary methods were developed to estimate turbidity. Electronic nephelometers are the preferred instruments for turbidity measurement.

Most commercial turbidimeters designed for measuring low turbidities give comparatively good indications of the intensity of light scattered in one particular direction, predominantly at right angles to the incident light. Turbidimeters with scattered-light detectors located at 90° to the incident beam are called nephelometers. Nephelometers are relatively unaffected by small differences in design parameters and therefore are specified as the standard instrument for measurement of low turbidities. Instruments of different make and model may vary in response. † However, interinstrument variation may be effectively negligible if good measurement techniques are used and the characteristics of the particles in the measured suspensions are similar. Poor measurement technique can have a greater effect on measurement error than small differences in instrument design. Turbidimeters of nonstandard design, such as forward-scattering devices, may be more sensitive than nephelometers to the presence of larger particles. While it may not be appropriate to compare their output with that of instruments of standard design, they still may be useful for process monitoring.

An additional cause of discrepancies in turbidity analysis is the use of suspensions of different types of particulate matter for instrument calibration. Like water samples, prepared suspensions have different optical properties depending on the particle size distributions, shapes, and refractive indices. A standard reference suspension having reproducible light-scattering properties is specified for nephelometer calibration.

Its precision, sensitivity, and applicability over a wide turbidity range make the nephelometric method preferable to visual methods. Report nephelometric measurement results as nephelometric turbidity units (NTU).

3. Storage of Sample

Determine turbidity as soon as possible after the sample is taken. Gently agitate all samples before examination to ensure a representative measurement. Sample preservation is not practical; begin analysis promptly. Refrigerate or cool to 4°C, to minimize microbiological decomposition of solids, if storage is required. For best results, measure turbidity immediately without altering the original sample conditions such as temperature or pH.

* Approved by Standard Methods Committee, 2001. Editorial revisions, 2011. Joint Task Group: 20th Edition—Raymond D. Letterman (chair), John A. Arrington, Alvin Lieberman, Kemon J. Papacosta, Theodore S. Tanaka, Brannon H. Wilder.

† Nephelometers that instrument manufacturers claim meet the design specifications of this method may not give the same reading for a given suspension, even when each instrument has been calibrated using the manufacturer's manual. This differential performance is especially important when measurements are made for regulatory purposes. Consult regulatory authorities when selecting a nephelometer to be used for making measurements that will be reported for regulatory purposes.

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2130 B. Nephelometric Method

1. General Discussion

a. Principle: This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity. Formazin polymer is used as the primary standard reference suspension. The turbidity of a specified concentration of formazin suspension is defined as 4000 NTU.

b. Interference: Turbidity can be determined for any water sample that is free of debris and rapidly settling coarse sediment. Dirty glassware and the presence of air bubbles give false results. "True color" (i.e., water color due to dissolved substances that absorb light) causes measured turbidities to be low. This effect usually is not significant in treated water.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

2. Apparatus

a. Laboratory or process nephelometer consisting of a light source for illuminating the sample and one or more photoelectric detectors with a readout device to indicate intensity of light scattered at 90° to the path of incident light. Use an instrument designed to minimize stray light reaching the detector in the absence of turbidity and to be free from significant drift after a short warmup period. The sensitivity of the instrument should permit detecting turbidity differences of 0.02 NTU or less in the lowest range in waters having a turbidity of less than 1 NTU. Several ranges may be necessary to obtain both adequate coverage and sufficient sensitivity for low turbidities. Differences in instrument design will cause differences in measured values for turbidity even though the same suspension is used for calibration. To minimize such differences, observe the following design criteria:

1) Light source—Tungsten-filament lamp operated at a color temperature between 2200 and 3000K.
2) Distance traversed by incident light and scattered light within the sample tube—Total not to exceed 10 cm.
3) Angle of light acceptance by detector—Centered at 90° to the incident light path and not to exceed ±30° from 90°. The detector and filter system, if used, shall have a spectral peak response between 400 and 600 nm.

b. Sample cells: Use sample cells or tubes of clear, colorless glass or plastic. Keep cells scrupulously clean, both inside and out, and discard if scratched or etched. Never handle them where the instrument's light beam will strike them. Use tubes with sufficient extra length, or with a protective case, so that they may be handled properly. Fill cells with samples and standards that have been agitated thoroughly and allow sufficient time for bubbles to escape.

Clean sample cells by thorough washing with laboratory soap inside and out followed by multiple rinses with distilled or deionized water; let cells air-dry. Handle sample cells only by the top to avoid dirt and fingerprints within the light path.

Cells may be coated on the outside with a thin layer of silicone oil to mask minor imperfections and scratches that may contribute to stray light. Use silicone oil with the same refractive index as glass. Avoid excess oil because it may attract dirt and contaminate the sample compartment of the instrument. Using a soft, lint-free cloth, spread the oil uniformly and wipe off excess. The cell should appear to be nearly dry with little or no visible oil.

Because small differences between sample cells significantly impact measurement, use either matched pairs of cells or the same cell for both standardization and sample measurement.

3. Reagents

a. Dilution water: High-purity water will cause some light scattering, which is detected by nephelometers as turbidity. To obtain low-turbidity water for dilutions, nominal value 0.02 NTU, pass laboratory reagent-grade water through a filter with pore size sufficiently small to remove essentially all particles larger than 0.1 μm;* the usual membrane filter used for bacteriological examinations is not satisfactory. Rinse collecting flask at least twice with filtered water and discard the next 200 mL.

Some commercial bottled demineralized waters have a low turbidity. These may be used when filtration is impractical or a good grade of water is not available to filter in the laboratory. Check turbidity of bottled water to make sure it is lower than the level that can be achieved in the laboratory.

b. Stock primary standard formazin suspension:

1) Solution I—Dissolve 1.000 g hydrazine sulfate, $(NH_2)_2 \cdot H_2SO_4$, in distilled water and dilute to 100 mL in a volumetric flask. **CAUTION: Hydrazine sulfate is a carcinogen; avoid inhalation, ingestion, and skin contact. Formazin suspensions can contain residual hydrazine sulfate.**

2) Solution II—Dissolve 10.00 g hexamethylenetetramine, $(CH_2)_6N_4$, in distilled water and dilute to 100 mL in a volumetric flask.

3) In a flask, mix 5.0 mL Solution I and 5.0 mL Solution II. Let stand for 24 h at 25 ± 3°C. This results in a 4000-NTU suspension. Transfer stock suspension to an amber glass or other UV-light-blocking bottle for storage. Make dilutions from this stock suspension. The stock suspension is stable for up to 1 year when properly stored.

c. Dilute turbidity suspensions: Dilute 4000 NTU primary standard suspension with high-quality dilution water. Prepare immediately before use and discard after use.

d. Secondary standards: Secondary standards are standards that the manufacturer (or an independent testing organization) has certified will give instrument calibration results equivalent (within certain limits) to the results obtained when the instrument is calibrated with the primary standard (i.e., user-prepared formazin). Various secondary standards are available including: commercial stock suspensions of 4000 NTU formazin, commercial suspensions of microspheres of styrene-divinylbenzene co-

* Nuclepore Corp., 7035 Commerce Circle, Pleasanton, CA, or equivalent.

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polymer,† and items supplied by instrument manufacturers, such as sealed sample cells filled with latex suspension or with metal oxide particles in a polymer gel. The U.S. Environmental Protection Agency[1] designates user-prepared formazin, commercial stock formazin suspensions, and commercial styrene-divinylbenzene suspensions as "primary standards," and reserves the term "secondary standard" for the sealed standards mentioned above.

Secondary standards made with suspensions of microspheres of styrene-divinylbenzene copolymer typically are as stable as concentrated formazin and are much more stable than diluted formazin. These suspensions can be instrument-specific; therefore, use only suspensions formulated for the type of nephelometer being used. Secondary standards provided by the instrument manufacturer (sometimes called "permanent" standards) may be necessary to standardize some instruments before each reading and in other instruments only as a calibration check to determine when calibration with the primary standard is necessary.

All secondary standards, even so-called "permanent" standards, change with time. Replace them when their age exceeds the shelf life. Deterioration can be detected by measuring the turbidity of the standard after calibrating the instrument with a fresh formazin or microsphere suspension. If there is any doubt about the integrity or turbidity value of any secondary standard, check instrument calibration first with another secondary standard and then, if necessary, with user-prepared formazin. Most secondary standards have been carefully prepared by their manufacturer and should, if properly used, give good agreement with formazin. Prepare formazin primary standard only as a last resort. Proper application of secondary standards is specific for each make and model of nephelometer. Not all secondary standards have to be discarded when comparison with a primary standard shows that their turbidity value has changed. In some cases, the secondary standard should be simply relabeled with the new turbidity value. Always follow the manufacturer's directions.

4. Procedure

a. General measurement techniques: Proper measurement techniques are important in minimizing the effects of instrument variables as well as stray light and air bubbles. Regardless of the instrument used, the measurement will be more accurate, precise, and repeatable if close attention is paid to proper measurement techniques.

Measure turbidity immediately to prevent temperature changes and particle flocculation and sedimentation from changing sample characteristics. If flocculation is apparent, break up aggregates by agitation. Avoid dilution whenever possible. Particles suspended in the original sample may dissolve or otherwise change characteristics when the temperature changes or when the sample is diluted.

Remove air or other entrained gases in the sample before measurement. Preferably degas even if no bubbles are visible. Degas by applying a partial vacuum, adding a nonfoaming-type surfactant, using an ultrasonic bath, or applying heat. In some cases, two or more of these techniques may be combined for more effective bubble removal. For example, it may be necessary to combine addition of a surfactant with use of an ultrasonic bath for some severe conditions. Any of these techniques, if misapplied, can alter sample turbidity; *use with care*. If degassing cannot be applied, bubble formation will be minimized if the samples are maintained at the temperature and pressure of the water before sampling.

Do not remove air bubbles by letting sample stand for a period of time because during standing, turbidity-causing particulates may settle and sample temperature may change. Both of these conditions alter sample turbidity, resulting in a nonrepresentative measurement.

Condensation may occur on the outside surface of a sample cell when a cold sample is being measured in a warm, humid environment. This interferes with turbidity measurement. Remove all moisture from the outside of the sample cell before placing the cell in the instrument. If fogging recurs, let sample warm slightly by letting it stand at room temperature or by partially immersing it in a warm water bath for a short time. Make sure samples are again well mixed.

b. Nephelometer calibration: Follow the manufacturer's operating instructions. Run at least one standard in each instrument range to be used. Make certain the nephelometer gives stable readings in all sensitivity ranges used. Follow techniques outlined in Sections 2130B.2*b* and 4*a* for care and handling of sample cells, degassing, and dealing with condensation.

c. Measurement of turbidity: Gently agitate sample. Wait until air bubbles disappear and pour sample into cell. When possible, pour well-mixed sample into cell and immerse it in an ultrasonic bath for 1 to 2 s or apply vacuum degassing, causing complete bubble release. Read turbidity directly from instrument display.

d. Calibration of continuous turbidity monitors: Calibrate continuous turbidity monitors for low turbidities by determining turbidity of the water flowing out of them, using a laboratory-model nephelometer, or calibrate the instruments according to manufacturer's instructions with formazin primary standard or appropriate secondary standard.

5. Interpretation of Results

Report turbidity readings as follows:

Turbidity Range *NTU*	Report to the Nearest *NTU*
0–1.0	0.05
1–10	0.1
10–40	1
40–100	5
100–400	10
400–1000	50
>1000	100

When comparing water treatment efficiencies, do not estimate turbidity more closely than specified above. Uncertainties and discrepancies in turbidity measurements make it unlikely that results can be duplicated to greater precision than specified.

† AMCO-AEPA-1 Standard, Advanced Polymer Systems, 3696 Haven Ave., Redwood City, CA, or equivalent.

https://doi.org/10.2105/SMWW.2882.018

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Frequently Asked Questions Regarding Turbidity from Hach

What is the difference between the turbidity units NTU, FNU, FTU, and FAU? What is a JTU?

Problem Solution:

NTU stands for Nephelometric Turbidity Unit and signifies that the instrument is measuring scattered light from the sample at a 90-degree angle from the incident light. FNU standards for Formazin Nephelometric Units and also signifies that the instrument is measuring scattered light from the sample at a 90-degree angle from the incident light. NTU is most often used when referencing the USEPA Method 180.1 or Standard Methods For the Examination of Water and Wastewater. FNU is most often used when referencing the ISO 7027 (European) turbidity method.

When formazin was initially adopted as the primary reference standard for turbidity, units of FTU or Formazin Turbidity Units were used. These units, however, do not specify how the instrument measures the sample.

FAU or Formazin Attenuation Units signify that the instrument is measuring the decrease in transmitted light through the sample at an angle of 180 degrees to the incident light. This type of measurement is often made in a spectrophotometer or colorimeter and is not considered a valid turbidity measurement by most regulatory agencies.

The turbidity units NTU, FNU, FTU, AND FAU are all based on calibrations using the same formazin primary standards. Therefore when a formazin standard is measured, the value for each of these units will be the same, however the value on samples may differ significantly.

A JTU or Jackson Turbidity Unit is a historical unit used when measurements were made visually using a Jackson Candle Turbidimeter. Water was poured into a tube until a flame underneath the tube could no longer be distinguished.

Why do I need to calibrate my turbidimeter? Aren't turbidimeters calibrated at the factory?

Problem Solution:

The light source used in turbidimeters has a specified temperature (required by USEPA regulations) at which it is required to operate. Because these lamps operate at a relatively high temperature, the output of the lamps can change over time, which can affect the amount of light that reaches the detector and thus the turbidity reading.

Hach lamps are specially designed for increased stability and are stable upon factory calibration. However, USEPA regulations mandate calibration upon receipt and set up of the instrument at the test site, as well as calibration on a quarterly basis.

Can I use Gelex standards to calibrate my turbidimeter?

Problem Solution:

Gelex standards should never be used to calibrate a turbidimeter. They are designed to check the calibration after the turbidimeter has been calibrated with a primary standard such as StablCal.

After calibrating the turbidimeter with StablCal or formazin standards, place each Gelex vial in the sample compartment, and write the NTU value of each Gelex standard on each vial. Place the Gelex vials in the turbidimeter on a regular basis and compare the NTU value with the value written on the vial. When the measured turbidity is greater than 5 percent different from the recorded value, the turbidimeter should be calibrated again using a primary standard.

Note: you may need to calibrate your turbidimeter more often than indicated by the reading from the Gelex vials in order to meet regulatory compliance

How do I use Gelex secondary standards?

Problem Solution:

Gelex standards are secondary standards in solid or gel form that require no dilution or mixing. They provide a quick and convenient way to check if the calibration of laboratory and portable turbidimeters has changed on a daily basis. To use, place the Gelex standard in the sample cell compartment in a defined orientation right after the turbidimeter has been calibrated with a primary

standard, and record the displayed value. Then periodically (daily or weekly, for instance) place the standard back in the instrument in the same orientation to verify that the calibration has not changed significantly. When the value of the Gelex standard differs by more than 5% from the value right after the turbidimeter was last calibrated, the turbidimeter should be calibrated again with a primary formazin or StablCal standard. Both formazin and StablCal are accepted primary standards for turbidimeter calibration. Gelex standards should never be used to calibrate a turbidimeter, only to check the calibration after the turbidimeter has been calibrated with a primary standard.

My laboratory turbidimeter reads higher than my on-line turbidimeter on the same sample. Which instrument is correct?

Problem Solution:

When measuring very clean water with very low turbidity (below about 0.2 NTU), it becomes more and more difficult to obtain an accurate reading with a laboratory turbidimeter. This is primarily due to contamination of the sample cells and stray light in the instrument, which can be caused by imperfections on the surface of the sample cells as well as dust on the optical lenses and other internal components.

Sample cells must be cleaned meticulously with 1:1 hydrochloric acid and rinsed thoroughly with distilled or deionized water and then sample water. Sonicating the cells in a sonicating bath is also helpful for dislodging particles attached to the cell walls. Once clean, sample cells should be stored with the caps on and rinsed with deionized water immediately after sample measurement.

When contamination is removed from the sample cells and silicone oil is properly applied to minimize the effects of scratches on the outer cell walls, the turbidity levels will drop and approach the readings obtained from the process turbidimeter. Because process turbidimeters do not use sample cells and the detector is placed directly in the sample stream, turbidity due to contamination and stray light are minimized.

Our new turbidimeter reads higher than the one we've used for decades (for example 2100A turbidimeter). We prefer the readings from the older instrument. What can we do?

Problem Solution:

Different turbidimeters may have different light sources, photodetectors, and optical systems that can result in differences in sensitivity and linearity between instruments. In addition, the way that the zero point is set and how calibrations are done has changed over the years to improve accuracy, especially at low concentrations. Many older turbidimeters did not incorporate the ratio mode for color interference and therefore give lower than actual results for samples having color. Some of the older turbidimeters also had zero knobs to zero out the reading from deionized water. Modern instruments do not zero on deionized water because even the cleanest water will have some turbidity. Zero knobs create a false negative condition where the instrument displays a lower than actual result. Newer instruments use the dark current (90-degree detector current with the lamp off) reading, made when the instrument is first turned on, to set the zero point. A dilution water (or <0.1 NTU StablCal) reading is taken and used to adjust the concentration of calibration standards for greatest accuracy. Modern turbidimeters incorporate the latest advances in electronics, optical components, and design, and will have the lowest stray light interference and give the most accurate results.

My 2100N or 2100AN Laboratory Turbidimeter does not display 0.000 when no sample is in the instrument. Does it need repair?

Problem Solution:

The empty cell reading (no sample cell in instrument, lid closed) in a turbidimeter displays stray light in the instrument and is normal. Stray light comes from light that reflects off internal surfaces in the instrument and reaches the detector. Stray light increases over time as dust collects on the optical lenses and other surfaces.

When the stray light reading gets above 0.03 NTU, clean the EPA filter (Catalog No. 3031200) with glass cleaner and a lint-free cloth. If the reading is still high, the instrument should be thoroughly cleaned by the Hach Service Department.

2100N LABORATORY TURBIDIMETER QUICK REFERENCE GUIDE

NEPHELOMETRIC MEASUREMENT PROCEDURE

1. Collect a representative sample in a clean container. Fill the sample cell to the line (approximately 30 mL). Take care to handle the sample cell by the top. Cap the sample cell. (*Note: Instrument warm-up stabilization time with Ratio on is 30 minutes and with Ratio off is 60 minutes. Typical application is to leave the instrument on 24 hours a day.)*

2. Hold the sample cell by the cap, and wipe to remove water spots and finger prints.

3. Apply a thin bead of silicone oil from the top to the bottom of the cell—just enough to coat the cell with a thin layer of oil. Using the oiling cloth provided, spread the oil uniformly. Then, wipe off the excess. The cell should appear nearly dry with little or no visible oil. (*Note: See Section 2.3.2 Applying Silicone Oil in the instrument manual.)*

4. Place the sample cell in the instrument cell compartment, and close the cell cover. (*Note: For immediate update of the display, press* **ENTER.**)

5. If necessary, insert the EPA filter. Select manual or automatic ranging by pressing the **RANGE** key.

6. Select the appropriate **SIGNAL AVERAGING** setting (on or off) by pressing the **SIGNAL AVG** key.

7. Select the appropriate **RATIO** setting (on or off) by pressing the **RATIO** key. (*Note: Values >40 NTU require Ratio on*.)

8. Select the appropriate measurement unit (NTU, EBC or NEPH) by pressing the **UNITS/EXIT** key.

9. Read and record the results.

CALIBRATION

Preparing Recommended Formazin Dilutions

Hach Company recommends use of 20-, 200-, 1000- and 4000-NTU Formazin standards for calibration of the Model 2100N Turbidimeter. Prepare all Formazin dilutions immediately before calibration, and discard the dilutions after use. While 4000-NTU stock solutions are stable for up to one year, diluted solutions deteriorate more rapidly. Prepare dilutions of 20, 200 and 1000 NTUs according to the directions in *Table 2 (Formazin Standard Preparation)* in *Section 3* of the Instrument Manual. The dilution water also is used to make an initial blank measurement (refer to *Section 3.2 Calibration* in the Instrument Manual).

NOTE

The calibration is based on a first order linear equation consisting of up to three independent variables. Unpredictable results may occur if standards other than the recommended calibration points are used. The factory-suggested calibration points are those determined by Hach Company chemists and engineers to provide the best calibration accuracy. Use of standards other than those specified may result in less accurate calibrations.

Calibrating with Formazin Standards

The electronic and optical design of the 2100N Turbidimeter provides long-term stability and minimizes the need for frequent calibration. The three-detector ratioing optical system compensates for electronic and optical system variations between calibrations. When data is used for USEPA reporting, recalibrate at least every 90 days, or as stipulated by the regulating authority. Refer to *Section 3.2 Calibration* in the Instrument Manual.

1. Fill a clean sample cell to the line (≅ 30 mL) with dilution water. Wipe the cell clean and apply a thin film of silicone oil.

2. Place the sample cell into the cell holder, and *close the cell cover.*

3. Press the **CAL** key. The S0 annunciator lights. The NTU value of the dilution water used in the previous calibration is displayed.

4. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. This result is stored and used to compensate for the turbidity of the dilution water.

5. The instrument automatically increments to the next standard, displays the expected NTU value (e.g., 20.00 NTU), and the S1 annunciator lights. Remove the sample cell from the cell holder.

6. Fill a clean sample cell to the line with well-mixed, 20-NTU Formazin standard. Wipe the sample cell clean, and apply a thin film of silicone oil on its surface. Place it into the cell holder, and *close the cell cover.*

7. Press the **ENTER** key. The display counts down from 60 to 0, and makes a measurement. The instrument automatically increments to the next standard, the display shows 200.0 NTU, and the S2 annunciator lights. Remove the sample cell from the instrument.

8. Fill a clean sample cell to the line with well-mixed, 200-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it into the cell holder, and *close the cell cover.* Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The instrument automatically increments to the next standard, the display shows 1000 NTU, and the S3 annunciator lights. Remove the sample cell from the instrument.

9. Fill a clean sample cell to the line with well-mixed, 1000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and *close the cell cover.* Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The display automatically increments to the next standard, the display shows 4000 NTU, and the S4 annunciator lights. Remove the sample cell from the instrument.

10. Fill a clean sample cell to the line with well-mixed, 4000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and *close the cell cover.* Press the **ENTER** key. The instrument counts down from 60 to 0, and then makes a measurement. The display automatically increments back to the dilution water standard. The S0 annunciator lights, and the previously measured value of the dilution water is displayed.

11. Press the **CAL** key. The instrument makes calculations based on the new calibration data, stores the new calibration and returns the instrument to the measurement mode.

Reviewing the Calibration Sequence

Press the **CAL** key and then use the **UP ARROW** key to scroll through the standards to review calibration data currently in effect. If the instrument is connected to a printer, pressing the **PRINT** key prints all of the calibration data in effect. Press the **UNITS/EXIT** key to return to the operating mode without altering the current calibration data.

Using Gelex® Secondary Turbidity Standards

Periodically, as experience or regulating authorities indicate, verify the instrument calibration using Gelex Secondary Standards. If the reading in the range of use is not within 5% of the standard's assigned value, recalibrate using Formazin primary standards (refer to *Section 3.2.5 Using Gelex Secondary Turbidity Standards* in the Instrument Manual).

1. Calibrate the instrument with Formazin (refer to *Section 3.2 Calibration* in the Instrument Manual).

2. Verify that the instrument is set for the NTU mode, Ratio on and Automatic Ranging.

3. Thoroughly clean the outside of the Gelex vials, and apply a thin coating of silicone oil.

4. Place the lowest NTU Gelex Standard in the sample compartment with the triangle on the vial aligned with the index mark on the instrument sample compartment. Close the sample cell cover.

5. Press the **ENTER** key. Record the value displayed. Remove the standard from the instrument, and mark this value on the vial with a water soluble marker.

6. Repeat steps 3 through 5 for the other Gelex standards.

NOTE

Reassign new values to the Gelex standards each time the instrument is calibrated with Formazin.

ERROR CODES

Error codes may result from instrument malfunction or operator error. **Errxx** error codes are cleared from the display by pressing the **ENTER** key. The meter continues operating in the error condition; a calibration in progress can be continued. Any calibration being calculated (at the time the message appears) is discarded; the old calibration is retained. *Table 1* lists the error codes displayed for specific conditions.

Table 1. Error Codes

Code	Probable Cause	Corrective Action
Err01	Dilution water calculated to be >0.5 NTU	Start calibration over with higher quality dilution water, or filter the water with a membrane filter before use.
Err02	Two calibration standards have the same value, or their difference is less than 60.0 NTU. Standard 1 is too low (<10 NTU)	Recheck preparation of standards and repeat calibration.
Err03	Low light error	Reinsert sample. Check that lamp is on. Dilution may be necessary.
Err04	Memory malfunction	Switch instrument off and back on with I/O. Call Hach Service.
Err05	A/D over-range	Contact Hach Service.
Err06	A/D under-range	Contact Hach Service.
Err07	Light leak	Contact Hach Service.
Err08	Bad lamp circuit	Contact Hach Service.
Err09	Printer timeout error	Check that external printer is properly connected. Check that external printer is selected (on-line).
Err10	System voltage out of range	Switch instrument off and back on with I/O. Call Hach Service.
Err11	System loop test error	Switch instrument off and back on with I/O. Call Hach Service.

Diagnostic Functions

The diagnostic mode accesses system function information that is useful primarily when the instrument function is in doubt. Hach service technicians use the information for precise troubleshooting, speeding repairs, and avoiding unnecessary service returns.

Access diagnostic information by pressing and holding the **RIGHT ARROW** key for 3 seconds. Use the **ARROW** keys to edit the display to read the diagnostic code number of interest. Press the **ENTER** key to display the diagnostic value. More information may be obtained by purchasing the instrument service manual, or contacting the service center nearest you.

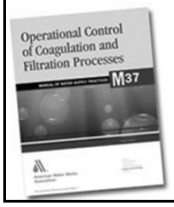
Diagnostic Codes

Code	Display	Description
00	bP on/bP of	Keyboard Beeper On/Off
01	FS Pr/SL Pr	Fast/Slow Print Device
21	Pr In	Printer Test
22	*	Display Test
23	*	Keyboard Test
24	*	Memory Test

Refer to *Table 6 Diagnostic Codes* in *Section 8 Troubleshooting* of the instrument manual for a list of diagnostic codes.

HACH COMPANY
WORLD HEADQUARTERS
P.O. BOX 389
Loveland, Colorado 80539
Telephone: (970) 669-3050
FAX: (970) 669-2932

Jar Testing



Water Lab Week 1

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TN Department of Environment & Conservation

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Water Treatment Processes

Fleming Training Center
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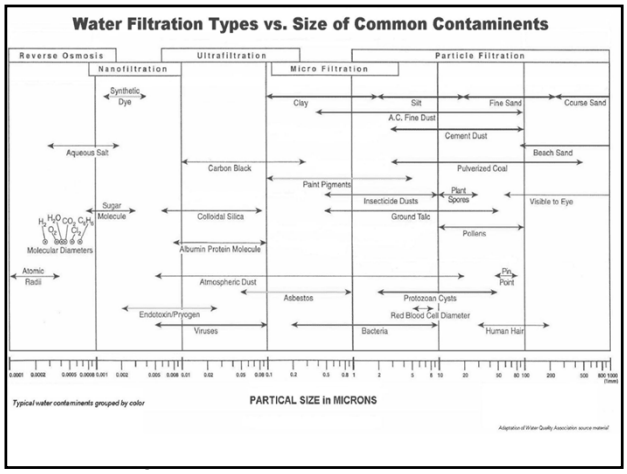
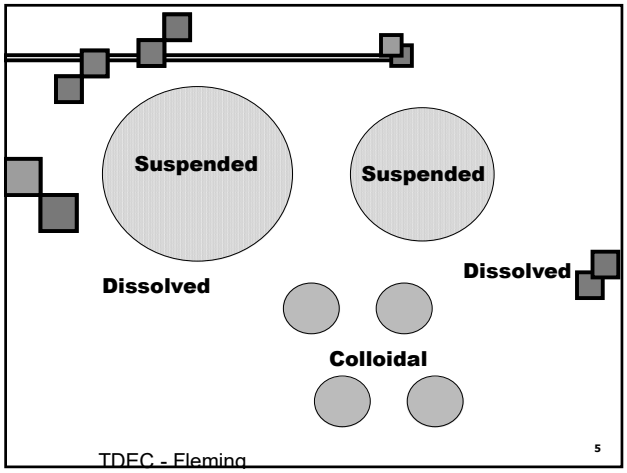
TDEC - Fleming 2

Objectives of Water Treatment

- One objective of water treatment professionals is to manage the removal of solids in the water that make it unsuitable for drinking.
- These solids can be either particulate, dissolved or colloidal in size and character.

TDEC - Fleming 3





TDEC - Fleming 5

Particle Settling Rate

Particle Diameter, mm	Representative Particle	Time Required to Settle 1 foot
10	Gravel	0.3 sec
1	Coarse sand	3 sec
0.1	Fine sand	38 sec
0.01	Silt	33 min
0.001	Bacteria	55 hours
0.0001	Color	230 days
0.00001	Colloidal particles	6.3 yrs
0.000001	Colloidal Particles	63 year min

TDEC - Fleming 6

Suspended Solid

- A solid organic or inorganic particle that is held in suspension by the action of flowing water.

Suspended Solids Common In Surface Waters

- Clay
- Sand
- Silt
- Bacteria
- Algae and other microorganisms

Suspended Solids Common In Surface Waters

- Effects of Particulates
  - Turbidity
  - Suspended solids
  - Taste and odors
  - Corrosion

Colloidal Solid

- Finely divided solid that will not settle out of water for very long periods of time unless the coagulation-flocculation process is used.

Colloidal Solids Common In Surface Waters

- Colored water
- Aquatic humic substances
- Decomposition by-products (organic)
- Industrial wastes
- Silica
- Calcium carbonate

Colloidal Solids Common In Surface Waters

- Effects of colloidal material
  - Turbidity
  - Deposition
  - Taste and odors
  - Increase coagulant usage and residuals
  - Disinfection by-products (THM's)

Dissolved Solid

- Any material that is dissolved in water and can be recovered by evaporating the water after filtering out the suspended material.

TDEC - Fleming 13

Dissolved Solids Common In Surface and Ground Waters

- Water hardness
  - Calcium
  - Magnesium
- Iron and manganese
- Heavy metals
- Nitrates and nitrites
- Salts
  - Chlorides
  - Sulfates
  - Sulfides
- Fluoride and silica compounds

TDEC - Fleming 14

Dissolved Solids Common In Surface and Ground Waters

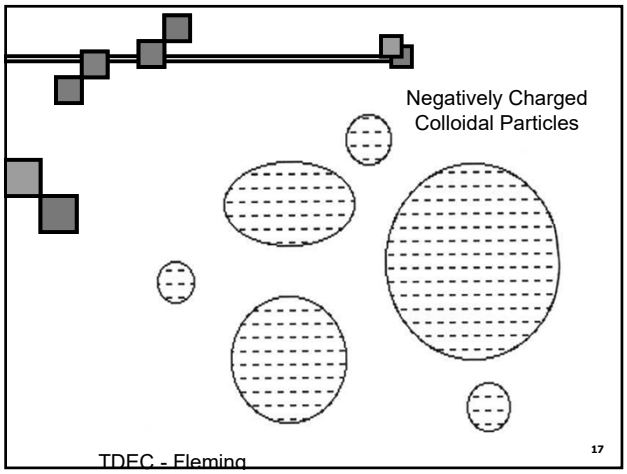
- Effects of dissolved solids
  - Scaling and deposits
  - Tastes and odors
  - Toxic effects
  - Corrosion
  - Staining

TDEC - Fleming 15

Colloidal and Suspended Particles

- These particles have a surface charge which is responsible for their "stability" to remain separate and suspended in the water.

TDEC - Fleming 16

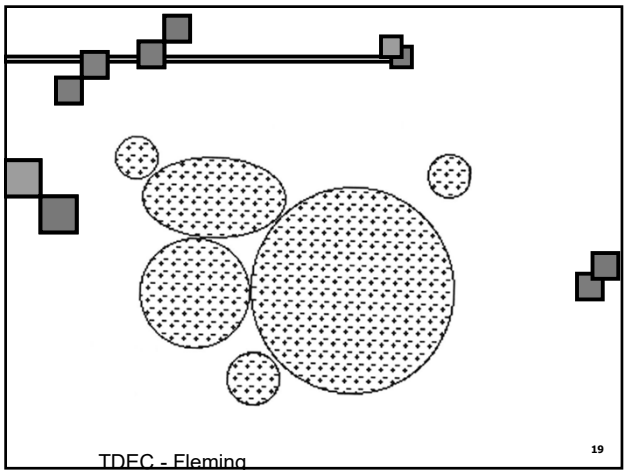


TDEC - Fleming 17

Coagulation

- The water treatment process that causes very small suspended particles to attract one another and form larger particles
- This is accomplished by the addition of a chemical, called a coagulant, that neutralizes the electrostatic charges on the particles that cause them to repel each other

TDEC - Fleming 18



Initial Mixing

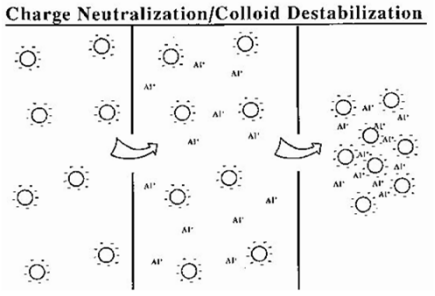
- Purpose is to completely mix the coagulant into the raw water flow stream in a fast and uniform manner
- Hydrolysis takes place almost instantaneously during the addition of the coagulant
  - Less than 1 second
- Complete floc development may take several minutes

Initial Mixing cont.

- Flash mix and rapid mix can be used interchangeably
- When conducting a jar test, the time the mixer runs at rapid mix should be equivalent to the effective detention time of the flash mix chamber in the plant

Flocculation

- The water treatment process, following coagulation, that uses gentle stirring to bring suspended particles together so that they will form larger, more settleable clumps called floc.



Floc Characteristics

- A typical conventional surface water treatment plant process that uses sedimentation followed by filtration benefits from a large, heavy floc that helps settling

Floc Characteristics cont.

- A common misconception is that increasing the chemical dose increases the settling efficiency of the floc particles because the larger floc looks great in the basins
  - This larger floc particle is more often just a chemical floc, such as aluminum hydroxide precipitate
  - In reality, this dose increase is a waste of chemicals and therefore money and it creates more sludge to waste

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Common Coagulants

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Coagulants

- Coagulants - positively charged chemicals that neutralize negatively charged particles in water to promote coagulation
  - Alum (aluminum sulfate)
  - Ferric Sulfate / Ferric Chloride
  - Organic Polymers
  - Polyaluminum chlorides

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Alum

- Advantages
  - Inexpensive
  - Handles variable water
  - Historically used coagulant
  - Broad application window
  - Effective at organic removal

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Alum

- Disadvantages
  - Produces large amounts of chemical solids
  - Reduction in alkalinity
  - Poor cold water performance
  - Potential post precipitation problems

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Ferric Sulfate/Ferric Chloride

- Advantages
  - Inexpensive
  - Applied over a broad pH range
  - Effective at organic removal
  - Large floc formation

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Ferric Sulfate/Ferric Chloride

- Disadvantages
  - Potential colored water
  - Highly staining chemical
  - Very corrosive products that can increase maintenance costs
  - Large chemical solids formation
  - Strong alkalinity reduction
  - Heavy metal considerations

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Polyaluminum Chloride Products

- Other chemical names
  - Aluminum Chlorohydrate (ACH)
  - Aluminum Hydroxychloride
  - Aluminum Chloride Hydroxide
  - Polyaluminum Chloride
  - Polyaluminumchlorosulfate

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Polyaluminum Chloride Products

- Advantages
  - Variety of products that can meet multiple goals
  - Improve overall water quality versus traditional coagulants
  - Better plant operations
  - ACH is highly concentrated aluminum source
  - Overall treatment cost savings

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Polyaluminum Chloride Products

- Disadvantages
  - Must base product choice on performance
  - Relative $/# is higher than traditional salts
  - Overall cost picture must be calculated
  - Must be applied properly
  - Most plants don't have historical data with PACl
  - Should match flocculant to PACl

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Organic Polymers

- Cationic polymers
  - Release positively charged ions
- Anionic polymers
  - Release negatively charged ions
- Nonionic polymers
  - Release both negatively and positively charged ions

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Organic Polymers

- Advantages
  - Low dosages applied
  - Minimal chemical solids generation
  - No alkalinity reduction
  - Aids in dewatering of sludge
  - Reaction independent of pH and alkalinity
  - Good cold water performance

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Organic Polymers

- Disadvantages
  - Expensive
  - Must base product choice on performance
  - Poor primary coagulants
  - Potential filter fouling
  - Can adversely effect filter run lengths
  - Possible monomer residuals

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Various Coagulant Goals

- Reduced particle counts
- Ease of use
- Cost savings
- Chemical residue (sludge) reduction
- Aluminum residual reduction
- Stable treated water
- Longer filter runs
- Cold water treatment
- TOC, THM, HAA removal
- Faster settling rates

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Various Coagulant Goals

- Set/prioritize treatment goals
- Understand individual raw water issues
  - Cold water
  - Low alkalinity
  - High organic episodes
  - Extremely variable turbidity water
  - Highly colored water
  - Easy to treat, constant water

52

Selecting the Correct Polymer

- Each polymer has a specific function
- Each type of polymer has benefits and limitations
- Understand it's limitations

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Water Soluble Solution Cationic Polymers

- Polymer reacts with the colloidal material in the water by neutralizing the charge or by bridging (tying together) individual particles to form visible insoluble floc
- Cationic polymers have a positive charge, and the colloidal material has a negative charge

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Define the Polymer Primary Treatment Goals

- Solids removal
- Settled turbidity
- Filtered turbidity
- Sludge reduction
- Filter run lengths
- Color removal

55

Understanding the Water Source

- Type of source
  - Well, Lake, River
- Parameters
  - Temperature
  - Turbidity
  - pH
  - Alkalinity

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Planning a Jar Test

TDEC - Fleming

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How to Run a Successful Plant Trial

- Define primary goals
- Understanding water source
- Chemical programs
- Design a chemical program
- Jar testing
- Plant trial

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Defining Primary Treatment Goals

- Solids removals
- Settled turbidity
- Filtered turbidity
- Color removal
- TOC/HAA removal
- Lengths of filtered runs
- Sludge reduction

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Chemical Program

- Each chemical used in a water plant influences and/or impacts the performance of another
- Understand its purpose and its limitations
- Design chemical program to:
  - Water Source
  - Applications
  - Goals

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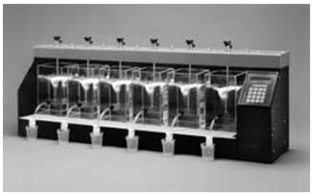
Jar Testing



- Used to screen different technologies
- Test procedure should correlate to plant's performance
  - Mixing energy
  - Slow mixing
  - Settling time

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Jar Testing

- Compare to plant's chemical program:
  - Settled turbidity
  - Filtered turbidity
  - pH
  - Alkalinity
  - Filtered Index
  - TOC/UV

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Planning and Scheduling

- Plan carefully
- Set clear goals
- Scheduling
- Training
- Polymer/coagulant introduction
- Equipment
- Technical support
- Measurements made

Note: Valid trials are an exercise in experimental design.

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Planning and Scheduling cont.

- Schedule the plant trial to avoid plant projects focus on trial
- Make the trial long enough 30 to 60 days
- First week for transition period
- Second week allows for dosage optimization and operators to become familiar with the program's strengths and weaknesses

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Planning and Scheduling cont.

- Data collected after this break in period provides a valid comparison to historical data

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Product Changeover

- Use a conservative approach
- Gradual introduction of new chemistry
- Gradual weaning off of old chemistry
  - 75/25% - 50/50% - 25/75% - 100%
- Use conservative dosage
  - Optimize later
- Adjust other chemistries systematically

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Operator Training

- The fate of any trial rests in the hands of the operators
- Operators should know the trial goals, understand the new chemistry, and what to expect

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Laboratory Testing Techniques

Equipment
Solution preparation
Test procedures

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Equipment

- Jars
  - Square
  - Round
- Stirrer
- Water bath
- Analytical Equipment
  - Turbidimeter
  - pH meter
  - Thermometer

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1-Liter Circular Jars

- Disadvantages:
  - Holds very little water
    - Minor errors in chemical doses result in large error in actual doses
  - Water rotates with paddles
    - Reduces the amount of actual mixing
  - Limited amount of water for analysis
  - Does not provide a good sampling point

In general, 1-L jars are not acceptable

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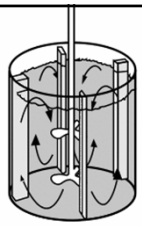
2-Liter Circular Jars



- Provides enough sample
- Stators inserted may help with mixing
  - May interfere with dosing and sampling
- Sample siphon can be added, but with some difficulty
- Disadvantage:
  - Water still rotates with the stirrer unless stators are used

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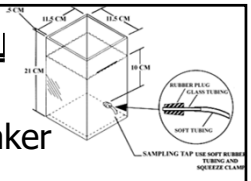
2-Liter Square Beaker



- Advantages:
  - Square configuration reduces rotation of water
  - Thicker wall and lower heat conductivity of plexiglass reduces water temp change during test
  - Jars are less fragile and can be repaired
  - Sampling port can easily be installed

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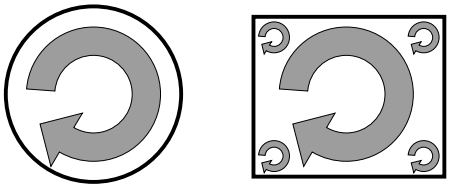
Round vs. Square Mixing



Use the same shape as your flash mix

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Stirrer

- Two basic types
  - Gear-driven
    - Best
  - Magnetic-driven
    - Easier to add chemicals
    - Doesn't turn smoothly at slow speeds

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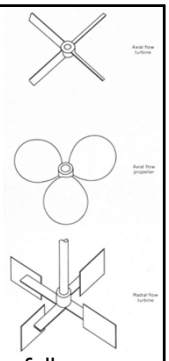
Impellers



- Types
  - Paddle
  - Turbine
  - Marine
  - Axial flow
- Need the one that best simulates full scale

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Impellers

- Magnetic stirrers are only available with paddles
  - Corresponds to paddle, walking-beam, flat-blade turbine type mixing devices
- Top mounted stirrers with flat paddles are best
  - Shaft and impeller can be changed

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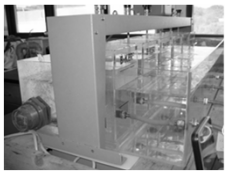
Water Bath

- Jars set in a rectangular tank with raw water circulating around them
- This is only necessary for cold water



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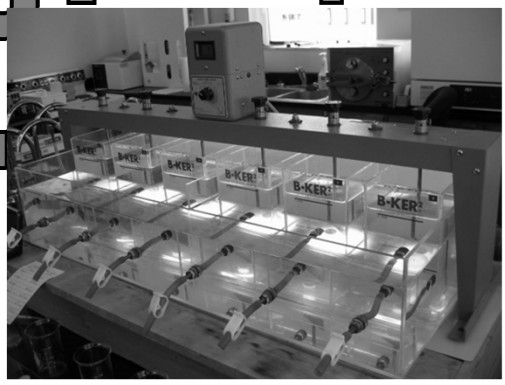

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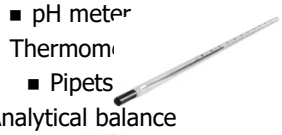
Water Bath

- Determination for use of water bath:
  - Take a raw sample
    - Run jar test immediately
  - Take another sample
    - Let warm 5-10 degrees
    - Run same test and take note in difference

81

Analytical Equipment

- Turbidimeter
- pH meter
- Thermometer
- Pipets
- Analytical balance



Solution Preparation

- Accuracy is critical - Small errors are compounded by large dilutions
- Dilute solutions of 1 g/L or less of coagulants or polymers should be prepared daily

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Solution Prep - Liquid Products

- Micropipets dispense neat product
- Specific gravity must be accounted for (liquid chemicals weigh more than water, so 1 mL of product weighs more than 1 gram)

$$\frac{mg/L}{sp.grav} = \mu L\ product\ /\ liter\ of\ test\ sol'n$$

* multiply x 2 for a 2 liter jar

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Solution Prep - Liquid Products

- For dose on a dry active product basis, divide by concentration:

$$\frac{mg/L}{(sp.grav)(conc)} = \mu L\ product/L\ test\ sol'n$$

* multiply x 2 for a 2 liter jar

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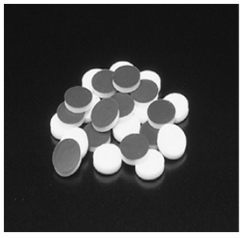
Micro-pipeting

- Try to place dose on an object that can perch on the rim of the jar until you are ready to add all at the same time
  - Septas from TOC vials
  - Powder pillows



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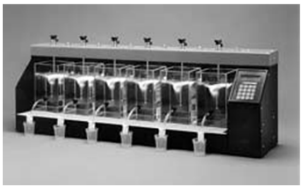
Jar Testing



- Must know volume of test jars and speed rates of stirrers prior to test
- Don't use laboratory grade chemicals
  - Use what you feed in plant

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Test Procedure

- Obtain fresh sample of water
- Sample temp. should not vary more than 3°C
- Record the following:

Temperature	pH	Turbidity
Color	Iron	Manganese
Hardness	Alkalinity	Fluoride
Phosphorus	Suspended Solids	

- Dispense equal volumes of water into jars

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Test Procedure

- Start mixing sequence and add test chemicals
- Always run a control of current program
- Mixing times and speeds are obtained from plant designs and operations
- Observe formation rate, floc size and settling rate
- Record all results

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Filterability Index

- Comparison of filtration rate of sample vs. DI water
- Helps predict the effect of a coagulant program on the filtration process
- Samples tested are clarified settled water, filter influent and jar test decants

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F-I Equipment

- Membrane filter holder 47mm
- Membrane filters 0.45 micrometers (μm)
- Vacuum source - consistent
- Filter flask, vacuum tubing, forceps
- Stopwatch
- Deionized water

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F-I Procedure

- Measure 200 mL DI water
- Place membrane in filter holder
- Start vacuum
- Add DI water
- Time in seconds to filter DI water
- Discard membrane and DI filtrate
- Repeat for test sample
- Test sample filtrate for water quality

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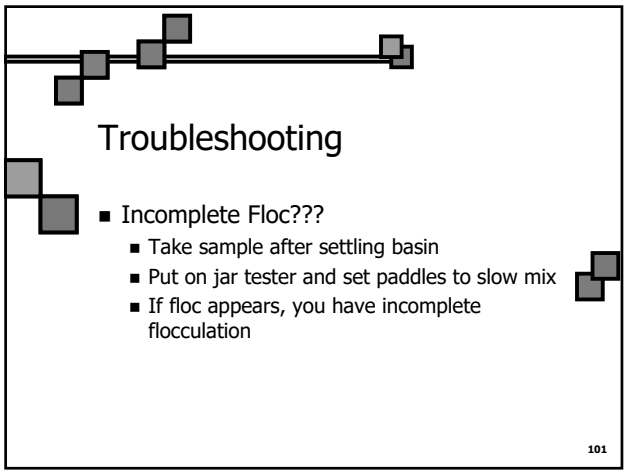
F-I Procedure

- Filterability Index (FI) = $\frac{\text{Time of Sample}}{\text{Time of DI water}}$
- Good Performance
  - Sand Filters FI < 3
  - High Rate Filters FI < 2
  - Filtered Turbidity < 0.3 NTU

100


Troubleshooting
Incomplete Floc???
Take sample after settling basin
Put on jar tester and set paddles to slow mix
If floc appears, you have incomplete flocculation
101

Jar Test Lab Math

1. The average flow for a water plant is 3.25 MGD. A jar test indicates that the best alum dosage is 2.5 mg/L. How many pounds per day will the operator feed?

2. Determine the setting on a dry alum feeder in pounds per day when the flow is 1.3 MGD. Jar tests indicate that the best alum dose is 12 mg/L.

3. A water treatment plant used 27 pounds of cationic polymer to treat 1.6 million gallons of water during a 24-hour period. What is the polymer dosage in mg/L?

4. Liquid polymer is supplied to a water treatment plant as an 8% solution. How many gallons of this liquid polymer should be used to make 200 gallons of a 0.7% polymer solution?

5. Liquid alum delivered to a water treatment plant contains 642.3 milligrams of alum per milliliter of liquid solution. Jar tests indicate that the best alum dose is 8 mg/L. Determine the setting on the liquid alum chemical feeder in milliliters per minute if the flow is 2.2 MGD.

6. A water plant is treating 8.2 MGD with 2.0 mg/L liquid alum. How many gpd of liquid alum will be required? The liquid alum contains 5.36 lbs dry alum/gallon.

7. An operator has decided to switch from dry alum to liquid alum. If he feeds an average of 100 lbs of dry alum a day, how many gallons of liquid alum will he need to feed on average given the following information:

Alum, liquid:	42.24% concentration
	11.07 lbs/gallon
	6.38 lbs dry alum/gallon
	1.3278 Specific Gravity

Coagulation/Flocculation Workshop
Conducting a Jar Test Study

Before starting:

- Define study goals
- Define testing parameters
- Have testing and analytical equipment ready
  - Working and clean
- Prepare reagent solutions
  - Properly labeled and mixed
- Data sheet available
  - Should be a good guide for conducting

Step 1. Find the specs for **full-scale plant**, including:

- Flash (rapid) mix
  - Dimensions (length x width x depth)
  - Type of mixing mechanism
    - mechanical, baffle, static mixer, etc.
  - Horsepower of mechanical mixer OR Hydraulic drop
  - Detention time (seconds)
  - Mixing Intensity (sec^{-1})
    - Velocity Gradient
- Flocculation basins
  - # of trains of basins
  - # of basins in series
  - For each basin in the series:
    - Dimensions (length x width x depth)
    - Type of mixing mechanism (mechanical, baffle, static mixer, etc.)
    - Horsepower of mechanical mixer OR Hydraulic drop
    - Detention time (in minutes)
    - Mixing Intensity (sec^{-1})
    - Velocity Gradient

- Sedimentation basins
    - Dimensions (length x width x depth)
    - Surface Overflow Rate (gpd/ft^2)
    - Settling Velocity (cm/min)
    - Sampling Time (min)
- All chemicals fed as part of treatment process
    - Chemical name
    - Chemical dosage
    - Chemical specific gravity
    - % concentration
    - Order of application

Step 2. Prepare jar test solutions. See handout "How To Make Dosing Solutions For Jar Tests"

- Sodium permanganate
- GPAC (inorganic coagulant)
- Clarifloc (coagulant aid)
- Lime (calcium hydroxide)

Step 3. Determine dosages for each chemical in each jar. Fill in datasheet (bench sheet).

- First jar should represent underdosing. Last jar should represent overdosing. Control jar (represents actual plant) should be in the middle.
- **Remember to only change one parameter at a time. All others remain constant.
    - This would be the time to decide what your Jar Test Study goals are. Based on that, you can choose which process parameter to alter. Examples of goals:
        - Organics removal
        - Removal of turbidity
        - pH adjustment
        - Hardness removal
        - Alkalinity additions

Step 4. Set up jar test machine and fill in datasheet.

- Rinse jars and paddles with DI water.
- Fill all jars with tap water. Lower paddles being sure to tighten the set screw.
- Turn jar test machine to maximum setting. Record RPMs on datasheet.
    - Determine Jar Test Time Correction. Record on datasheet.
- Complete remainder of datasheet to reflect entire process, including
    - Flocculation RPMs
    - Flocculation detention times
    - Sampling time

Step 5. Collect sample and run preliminary lab tests.

- Make sure sample is representative of the water in the plant. Compare pH in flash mix to pH of sample water.
- Be sure to collect enough sample to completely fill at least six 2-liter jars.
- Run all necessary lab tests on sample (mix sample well before each sub-sample collection).
    - pH
        - Calibrate probe; verify with 7.
    - Temperature
        - Can be read when measuring pH.
    - TOC
        - Instructor will run this for the class.
    - Turbidity
        - Calibrate turbidimeter if needed; otherwise, verify.
    - Alkalinity
        - Run Total Alkalinity using pH method.

Step 6. Run jar test. Use the datasheet as road map.

Step 7. Collect a sample from each jar. After filtering samples through a 45 µm filter, perform the same tests on the treated samples as was performed on the untreated water.

- Be sure to flush approximately 20 mL through the sample line before collecting sample to be tested.
- Compare before results and after results.
    - Determine % removal.
- Compare control to other jars.

Step 8. Record conclusions.

Jar Test Bench Sheet

Date:		
Time:		Concentration (mg/L)
Coagulant:		
Oxidant:		
Polymer:		

Source Water				
pH	Turbidity (ntu)	Alkalinity (mg/L)	Hardness (mg/L)	TOC (mg/L)

Jar Number		1	2	3	4	5	6
Rapid Mix	G (s^{-1})						
	rpm						
	Duration (s)						
Flocculator #1	G (s^{-1})						
	rpm						
	Duration (s)						
Flocculator #2	G (s^{-1})						
	rpm						
	Duration (s)						
Coagulant Dose (mg/L)							
Volume of Coagulant Added (mL)							
Polymer Dose							
Volume of Polymer Added (mL)							
Lime Dose (mg/L)							
Volume of Lime solution added (mL)							
Oxidant Dose (mg/L)							
Volume of Oxidant Added (mL)							
Settling Velocity (cm/min)	Depth of Sampling (cm)	Time of Settling (min)					
	10 cm						

Coagulation pH							
Record final jar test numbers here	pH						
	Turbidity						
	TOC						
	Alkalinity						
	Hardness						

Organic Carbon, Total

DOC316.53.01334

USEPA[1] Direct Method — Method 10267

1.5 to 30.0 mg/L C (LR) — TNTplus 810

Scope and application: For wastewater, drinking water, surface water and process water analyses.

[1] Hach Method 10267 is USEPA approved for the determination of total organic carbon (TOC) in drinking water, Federal Register Volume 81, Number 138 (Tuesday, July 19, 2016).

Test preparation

Before starting

DR 3900, DR 3800, DR 2800: Install the light shield in Cell Compartment #2 before this test is started.
Review the safety information and the expiration date on the package.
Use the DRB reactor with 13-mm wells for the digestion. If the reactor has 16-mm wells, insert adapter sleeves into the wells.
Make sure to digest the samples at 100 °C. Higher temperatures may cause the vials to break apart.
Be careful with the vials after the digestion. Pressure increases in the vials during the digestion and can cause the vials to break apart.
Use only the TOC-X5 shaker to remove total inorganic carbon (TIC) from the sample.
Carbon dioxide from the air can contaminate the sample. Do not open the indicator vial before the shaker operation is complete. Immediately install the double cap on the indicator vial after the cap is removed, then immediately install the other side of the double cap on the sample vial.
The formation of crystals in the sample vial does not affect the result.
The recommended temperature for reagent storage is 2–8 °C (35–46 °F).
The recommended sample pH is 3–10.
If the sample contains particles, dilute the sample. Use the diluted sample in the test procedure. Multiply the test result by the dilution factor.
After both vials are attached to the double cap, keep the vial assembly together. Put the vial assembly in the plastic packaging after the analysis.
DR 1900: Go to All Programs>LCK or TNTplus Methods>Options to select the TNTplus number for the test. Other instruments automatically select the method from the barcode on the vial.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

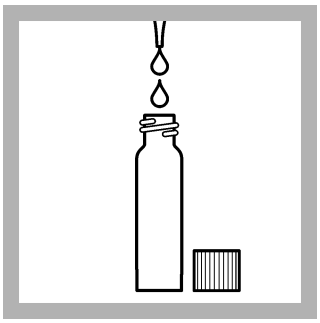
Description	Quantity
Total Organic Carbon, LR TNTplus 810 Reagent Set	1
DRB200 reactor with 13-mm wells	1
TOC-X5 shaker	1
Pipet, adjustable volume, 1.0–5.0 mL	1
Pipet tips, for 1.0–5.0 mL pipet	1
Test tube rack	1

Refer to Consumables and replacement items on page 4 for order information.

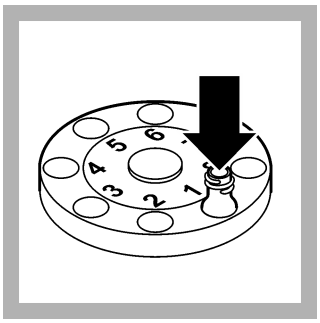
Sample collection

- Collect samples in clean glass bottles.
- Homogenize samples that contain solids to get a representative sample.
- Rinse the sample bottle several times with the sample to be collected.
- Fill the bottle completely full, then tighten the cap on the bottle.
- Analyze the samples as soon as possible for best results.
- Acid preservation is not recommended.

Test procedure

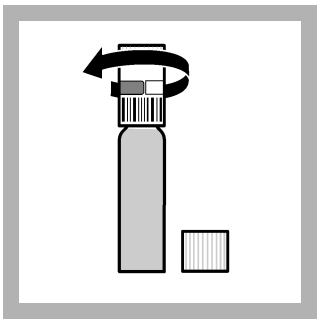


1. Remove the cap from a clear vial. Use a pipet to add 2 mL of sample to the vial.

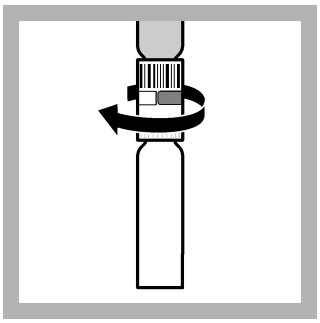


2. Insert the uncapped sample vial into the TOC-X5 shaker. Make sure that the vial is pushed all the way down into the shaker. Move the fan over the vial.

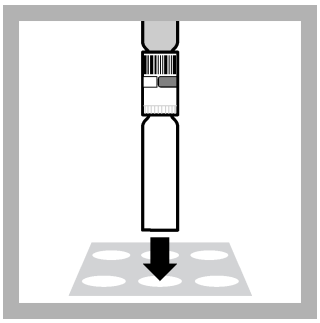
3. Push the on/off switch to start the shaker. Operate the shaker for 5 minutes.



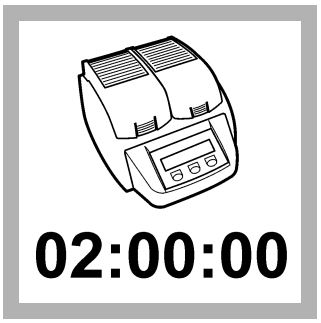
4. When the shake time is complete, remove the cap from a blue indicator vial. Immediately install and tighten a double cap on the indicator vial with the barcode label toward the vial.



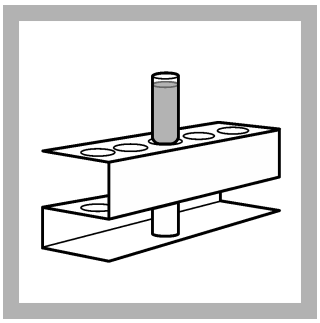
5. Immediately invert the indicator vial, then install and tighten the other side of the double cap on the sample vial. Hold the vial assembly vertically.



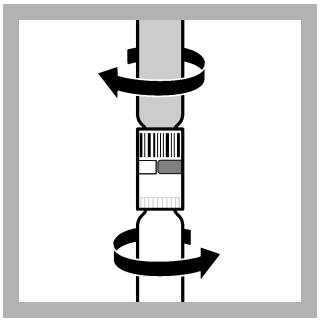
6. Insert the vial assembly into the DRB reactor (indicator vial on top).



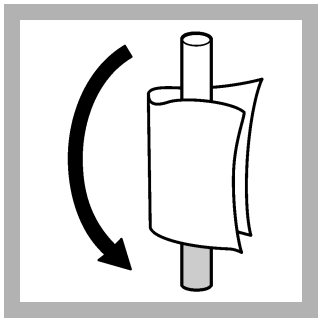
7. Increase the vial assembly temperature for 2 hours at 100 °C.



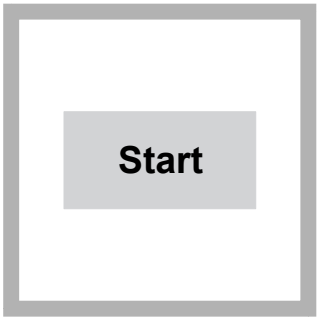
8. Let the vial assembly cool completely to room temperature. Make sure that the vials cool completely. Warm vials will give high results.



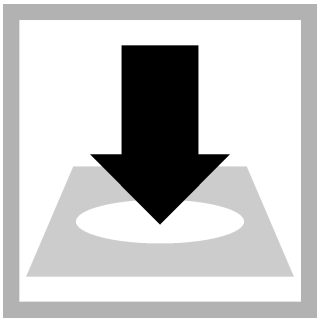
9. Tighten the double cap on both vials.



10. Invert the vial assembly so the indicator vial is on the bottom. Clean the indicator vial.



11. DR 1900 only: Select program 810. Refer to Before starting on page 1.



12. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in mg/L C.

Interferences

The table that follows shows the substances that were tested for interference and did not interfere up to the levels shown.

Interfering substance	Interference level
Ammonium	200 mg/L
Calcium	2000 mg/L as $CaCO_3$
Chloride	1000 mg/L
Magnesium	2000 mg/L as $CaCO_3$
TIC	250 mg/L

Accuracy check

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 1000-mg/L C TOC Standard Solution
- 500-mL volumetric flask, Class A
- 200-mL volumetric flask, Class A
- 50-mL volumetric pipet, Class A and pipet filler safety bulb
- 20-mL volumetric pipet, Class A and pipet filler safety bulb
- Organic-free water

1. Prepare a 100-mg/L C stock solution as follows:
   a. Use a pipet to add 20 mL of a 1000-mg/L C standard solution into a 200-mL volumetric flask.
   b. Dilute to the mark with organic-free water. Mix well.
2. Prepare a 10-mg/L C standard solution as follows:
   a. Use a pipet to add 50 mL of a 100-mg/L C stock solution into a 500-mL volumetric flask.
   b. Dilute to the mark with organic-free water. Mix well. Prepare this solution daily.
3. Use the test procedure to measure the concentration of the prepared standard solution.
4. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then*

used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
TNTplus 810	10 mg/L C	9.72–10.28 mg/L C	0.4 mg/L C

Summary of method

The total inorganic carbon (TIC) in the sample is first removed during the shaker operation. The sample is then digested to oxidize the total organic carbon (TOC) in the sample to carbon dioxide (CO_2). The CO_2 from the digested sample goes through the membrane in the double cap to the indicator vial and causes the indicator solution to change color. The color of the indicator solution is measured by the spectrophotometer. The measurement wavelength is 435 nm.

Consumables and replacement items

Description	Quantity/test	Unit	Item no.
Total Organic Carbon Reagent Set, LR, TNTplus	1	25/pkg	TNT810

Required apparatus

Description	Quantity/test	Unit	Item no.
DRB 200 Reactor, 115 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB200-01
DRB 200 Reactor, 230 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB200-05
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Test tube rack	1	each	1864100
TOC-X5 shaker	1	each	LQV148.99.00002
Wipes, disposable	1	280/pkg	2097000

Recommended standards

Description	Unit	Item no.
TOC Standard Solution Ampule (KHP Standard, 1000-mg/L C)	5/pkg	2791505

Optional reagents and apparatus

Description	Unit	Item no.
Reactor adapter sleeves, 16 mm to 13 mm diameter, for TNTplus vials	5/pkg	2895805
Ampule Breaker, 2-mL PourRite® Ampules	each	2484600
Flask, volumetric, Class A, 500 mL, glass	each	1457449
Flask, volumetric, Class A, 200 mL	each	1457445
Pipet, volumetric, Class A, 50 mL	each	1451541
Pipet, volumetric Class A, 20 mL	each	1451520
Pipet filler, safety bulb	each	1465100

Optional reagents and apparatus (continued)

Description	Unit	Item no.
Potassium Acid Phthalate (KHP), ACS	500 g	31534
Water, organic-free	500 mL	2641549

Organic Constituents, UV-Absorbing (UV-254)

DOC316.53.01092

Direct Reading Method[1]

Method 10054

Scope and application: For the determination of UV-absorbing organic compounds in drinking water and surface water.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*, Method 5910.

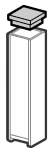
Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows requirements that can change between instruments, such as adapter and sample cell requirements.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information

Instrument	Adapter	Sample cell orientation	Sample cell
DR 6000	LZV902.99.00020 (universal) LZV902.99.00002 (1-cm carousel)	The clear side is to the right.	2624410 
DR 5000	A23618	The clear side is toward the user.	

Before starting

The sample pH must be 4 to 10 for accurate results. For SUVA calculations, do not adjust the sample pH.
Use a non-plastic filter assembly. Use a glass fiber filter of nominal pore size (1 to 1.5 µm) with no organic binder. For SUVA calculations, use a 0.45-µm filter and vacuum filtration apparatus.
Use only quartz sample cells for this test.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

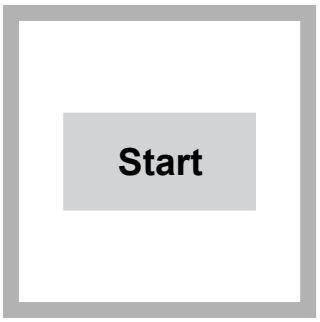
Description	Quantity
Organic-free reagent water	varies
Filter assembly	1
Buret stand	1
Sample cells (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1

Refer to Consumables and replacement items on page 4 for order information.

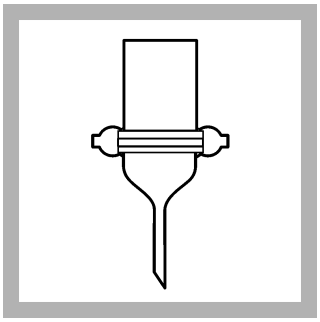
Sample collection

- Collect samples in clean glass bottles.
- Analyze the samples as soon as possible for best results.

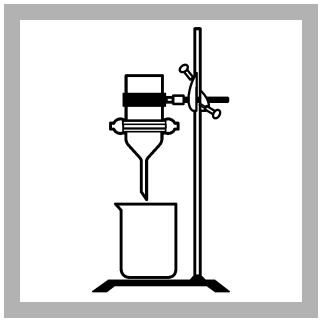
Test procedure



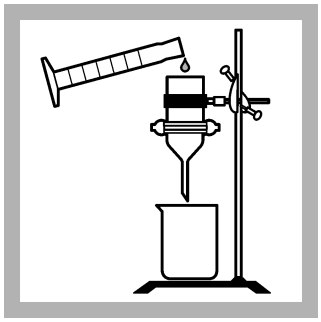
1. Start program **410 Organics, UV - 254**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



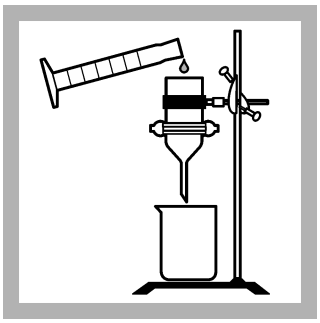
2. Assemble the filter apparatus. Make sure to use the white PTFE support plate. Insert the filter with the wrinkled surface pointed up.



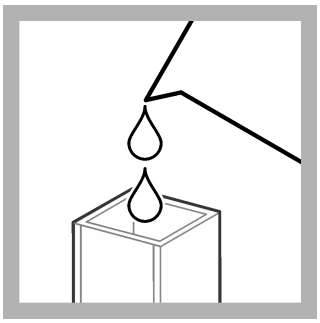
3. Install the apparatus in a support stand. Put a clean glass beaker below the filter.



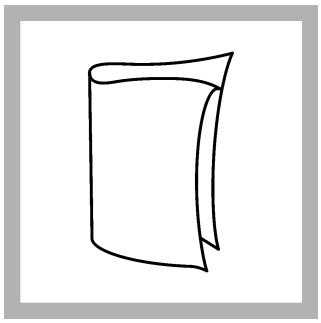
4. Prewash the filter assembly: Pour 50 mL of organic-free reagent water through the filter to remove soluble matter from the filter. Discard the filtered water.



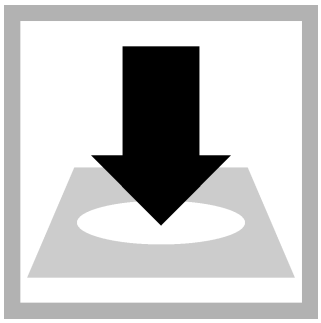
5. Prepare the sample: Pour 50 mL of sample through the filter.



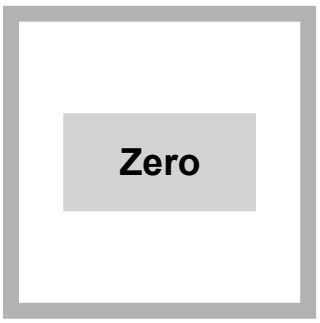
6. Prepare the blank: Rinse the sample cell several times with organic-free reagent water. Fill the sample cell with organic-free reagent water.



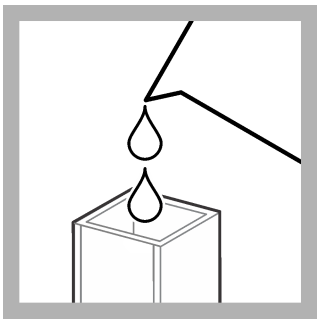
7. Clean the blank sample cell.



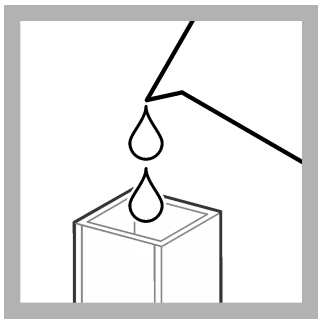
8. Insert the blank into the cell holder.



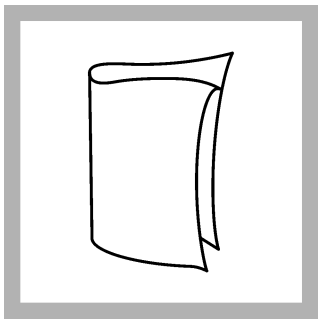
9. Push **ZERO**. The display shows 0.000 cm^{-1} and 1-cm cell. If necessary, wait 2 to 3 minutes for the Lamp Warm Up to complete.



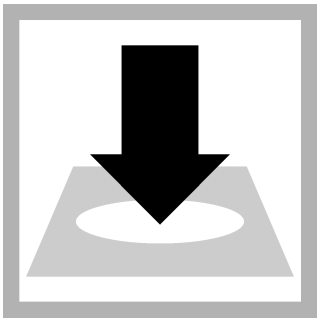
10. Discard the contents of the blank sample cell. Rinse the sample cell several times with the filtered sample.



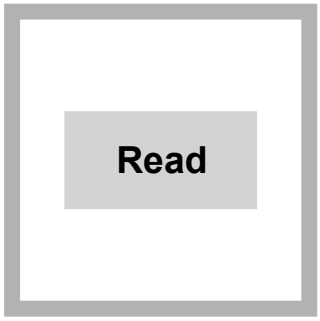
11. Fill the sample cell with the filtered sample.



12. Clean the sample cell.



13. Insert the sample cell into the cell holder.



14. Push **READ**. Results show in absorbance per centimeter (cm^{-1}).

Interferences

Interfering substance	Interference level
Extreme sample pH	Add 1 N Sodium Hydroxide or 1 N Sulfuric Acid to the sample to adjust the sample pH to 4 to 10.
UV-absorbing inorganics (bromide, ferrous iron, nitrate, nitrites)	Refer to Identify interferences in the sample on page 3.
UV-absorbing oxidants and reductants (chloramines, chlorates, chlorites, ozone, thiosulfates)	

Identify interferences in the sample

Do a baseline scan from 200 to 400 nm on the sample and the blank to identify if there are interferences in the sample.

1. From the main menu, push **WAVELENGTH SCAN>OPTIONS>λ**.
2. Push **200>OK**.
3. Push **400>OK**.
4. Push **1 NM>OK**.
5. Do steps 5 to 14 of the test procedure.
   If the sample scan shows sharp peaks, interferences may be in the sample.

Typically, natural organic material shows a featureless curve in the UV region with increased absorption as the wavelength decreases. If sharp peaks show, select and report a different wavelength.

Clean the sample cells

⚠WARNING

Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Refer to the current safety data sheets (MSDS/SDS) for safety protocols.

Clean new and dirty sample cells before use to remove all organic contamination. When the sample cells are rinsed every time with organic-free reagent water after use, it is only necessary to clean the sample cells occasionally.

1. Put the sample cells in 19.2 N Sulfuric Acid for a maximum of 12 hours.
2. Rinse the sample cells 10 times or more with organic-free reagent water.

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
410	—	0.431 to 0.433 cm^{-1}	—

Summary of method

The filtered sample is measured at 254 nm to show organic constituents in the sample water. Organic-free reagent water is used for the blank sample cell. Results are given in absorbance per centimeter (cm^{-1}). The results are used to calculate Specific Ultraviolet Absorbance (SUVA).

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
Organic-Free Reagent Water	varies	500 mL	2641549

Required apparatus

Description	Quantity/test	Unit	Item no.
Beaker, 100 mL	1	each	50042H
Buret stand	1	each	32900
Clamp holder	1	each	32600
Clamp, 3-prong	1	each	42200
Filter funnel assembly, 7-cm	1	each	2164100
Filter plate, PTFE, for filter funnel assembly	1	each	2164200
Filter, glass fiber, 70-mm	1	100/pkg	253053
Sample cell, 1-cm quartz	1	each	2624410

Optional reagents and apparatus

Description	Unit	Item no.
Cell holder for 10-cm sample cells (DR 5000 only)	each	LZY421
Sulfuric Acid Standard Solution, 19.2 N	500 mL	203849
Sodium Hydroxide Standard Solution, 1.00 N	100 mL MDB	104532
Sulfuric Acid Standard Solution, 1 N	100 mL MDB	127032
Filter pump, aspirator (SUVA)	each	213100
Filter, membrane, hydrophilic, polyethersulfone SUVA, 0.45 micron, 47 mm	100/pkg	2894700
Filter holder, glass for vacuum filtration (SUVA)	each	234000
Flask, filtering, glass, 1000 mL (SUVA)	each	54653
Graduated cylinder, 50 mL	each	50841
Paper, pH, 1.0–11.0	5 rolls/pkg	39133
Potassium Acid Phthalate (KHP), ACS	500 g	31534
Sample cells, 1-cm quartz matched pair	each	4822800

Optional reagents and apparatus (continued)

Description	Unit	Item no.
Sample cell, 5-cm rectangular quartz	each	2624450
Sample cell, 10-cm rectangular quartz	each	2624401
Standard Methods for the Examination of Water and Wastewater (current edition)	each	2270800
Tubing, latex rubber (SUVA), 5/16-in. inside diameter	3.66 m (12 ft)	56019