Microbiology

Acute waterborne diseases such as cholera and typhoid fever were major epidemics in the late-1800s and early-1900s. Methods to detect and remove these organisms were developed, and water operators are responsible to ensure the water supply is safe.

Diseases caused by pathogenic bacteria, viruses, and protozoa can be transmitted through fecal contamination to humans. Drinking water is just one of several carriers of these agents. Pathogens are disease-producing organisms and the presence of these is often related to poor sanitation practices. Microorganisms associated with recent waterborne outbreaks include the protozoa, bacteria, and virus. The following lists some of the most common waterborne diseases and their possible causes:

Waterborne Disease	Pathogen	Source of Pathogen	Symptoms
Gastroenteritis	Virus	Animal or Human Feces	Diarrhea, Vomiting
Typhoid Fever	Salmonella Typhosa	Human Feces	Inflamed Intestine, Enlarged Spleen, High Temperature
Dysentery	Shigella species	Human Feces	Diarrhea
Cholera	Vibrio Cholera	Human Feces	Vomiting, Severe Diarrhea, Dehydration
Infectious Hepatitis	Virus	Human Feces, Shell Fish	Yellowed Skin, Abdominal Pain
Amebic Dysentery	Entamoeba Histolitca	Human Feces	Mild Diarrhea
Giardiasis	Giardia Lamblia (a protozoa, one-celled animal)	Animal or Human Feces	Diarrhea, Cramps, Nausea, Weakness
Cryptosporidious	Cryptosporiduim parrum	Animal or Human Feces	Diarrhea, Cramps, Nausea, Weakness

It is difficult, expensive, and potentially hazardous to test for the presence of all types of pathogens. Instead, the technician or operator tests for the presence of indicators, organisms that, when present, indicate that pathogens may be present.

Coliforms have been chosen to be the bacterial group routinely tested to assess the bacteriological safety of water. Presence of any of the coliform group of bacteria, e.g, total coliforms, indicates general contamination, while the presence of Escherichia coli (E. coli) indicates contamination of human or animal origin. An ideal indicator organism would have the following characteristics:

- Indicator should always be absent in clean, uncontaminated water and present when pathogens are present.
- Indicator should be present in large numbers in fecal matter.
- Indicator and pathogen should respond similarly to treatment processes.
- Indicator should be easy to isolate, identify, and count.
- Ratio of indicator to pathogen should be high.
- Indicator and pathogen should come from the same source.

While total coliform do not meet all the criteria in all cases, they are the best indicators available.

TOTAL COLIFORM

The total coliform group of bacteria has been used as indicators in water treatment since the early 1900s. This group of organisms is found both in soil and in the waste of warm-blooded animals. It includes the Escherichia coli (E. coli), which is a common bacterium in the feces of warm-blooded animals.

Samples can be contaminated from external sources or there may have been other problems such as unsterile bottles and laboratory error. The most common problem involves errors in sampling. The current regulations require that if a sample is positive (shows the presence of coliforms), the water supplier must take four repeat samples, one at the same location as the sample that was positive, one within five service connections on each side of the positive location, and one from a representative site on the distribution system. For ground water systems, any source that was in use the week prior to the positive sample collection must be sampled.

The presence of E. coli provides stronger evidence of the possible presence of pathogens than do total coliform. If E. coli is detected in a sample, repeat samples must be collected as soon as possible or within 24 hours.

HETEROTROPHIC PLATE COUNT

The total or heterotrophic plate count (HPC) test measures the numbers present of a large group of bacteria, including both nonpathogens and pathogens. Because it does not isolate a specific organism, the HPC cannot be correlated with the likelihood of waterborne-disease outbreak. Water with a high HPC can contain many, few or no pathogens. The significance of using the HPC test is that it indicates a generally poor biological water quality for PWSs using surface water sources. Five-hundred colonies per milliliter have been suggested as an upper level, above which corrective action should be taken. This is applicable only to surface water supplies.

The microbiological contaminant section of the Public Water Supply Regulations chapter shows the frequency required.

SAMPLING AND SITE SELECTION

The number of samples required for the system is based on the size and number of people served by the system. Routine sampling sites should be representative of the water system. The sites should include some dead ends as well as areas where the flow is high. Sampling points may be scattered across the system and can include such sites as park buildings and fire stations. It is important that service lines are used and that the water being sampled comes directly from the water system rather than from the indoor plumbing.

Sampling taps should be selected carefully. Taps that should be avoided, if possible, include:

- Outdoor faucets with a likelihood of contamination from the ground surface. Frost-free hydrants should also be avoided since they can be contaminated by dust and snow.
- Mixing faucets where water from the hot side may not be representative of water from the system.
- Faucets supplying dishwater in cafes, drug stores, or other sites that may contain higher than normal bacterial contamination.
- Swing spouts because bacteria can grow where the faucet pivots.
- Leaky faucets or faucets that allow water to seep around the packing nut. A fixture in poor condition can introduce contamination into the sample.

SAMPLE CONTAINER

Proper use of the sample container is important. The sample can be positive for total coliform if the container becomes contaminated. Most laboratories supply sterilized sample bottles or bags to be used when taking the samples in addition to approved mailing cartons and appropriate forms. Sample containers may be made of glass or plastic.

- Bottles should have a wide mouth and a capacity of at least 125 milliliters. Only containers that are sterile and contain sodium thiosulfate to neutralize the chlorine in the water sample should be used. Most laboratories supply bottles that are ready for testing.

- Caps used on the sample must be sterile. Sample bottles should be examined for possible contamination. If the cap is loose, the bottle should not be used.

- Bottles that have been in storage for a long period of time have an increased likelihood of contamination and should not be used.

- Bottles without a label may get misplaced at the laboratory.

SAMPLE COLLECTION

1. **Sample Bottle/Preservative**: 150 milliliter (mL) sterile clear plastic Idexx bottle or 120 mL sterile clear plastic Idexx bottle . Preserved with sodium thiosulfate, a dechlorinating agent (do not rinse out).
2. **Shipping/Hold Time:** Ship immediately; sample analysis must begin within 30 hours of sample collection.
3. **Sample Locations:** The bacteriological sample must be taken at a location on the sample site plan or a representative location on the distribution system. Do not collect the sample from the well/pumphouse.
4. **Sample Collection Procedure**:

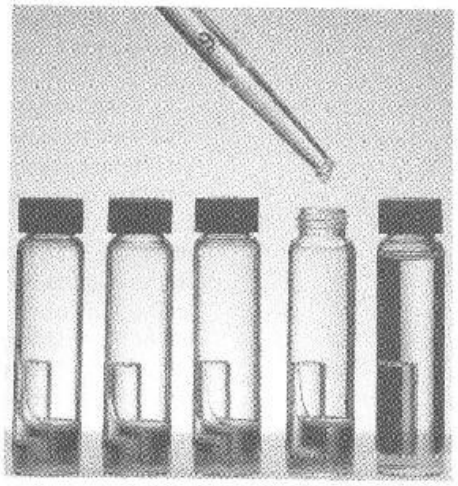
a. Remove any attachments on the faucet, including aerator, screens, washers, hoses, water purification devices. **OPTIONAL:** You may sterilize the faucet prior to collecting the sample. The sterilization is done after removing any attachments and before turning the faucet on. Flame the edge of the faucet with a torch or cigarette lighter for at least 15 seconds or wipe the edge with an alcohol wipe. IF FLAMING IS ATTEMPTED, DO SO WITH CAUTION. DO NOT FLAME PLASTIC FAUCETS.
b. Turn on the faucet and allow the water to run in a steady stream for 4 to 5 minutes. If sampling from a single lever faucet, be sure the handle is pushed over to the cold water side.
c. Turn the faucet down until a small smooth stream of water is running. DO NOT shut off the water.
d. Grasp the bottle around the lower half. Carefully remove the cap and hold the cap in hand. DO NOT touch the underside of the cap or inside the bottle. DO NOT flush out any liquid or powder that may be in the bottle, this is a dechlorinating agent.
e. Quickly place the bottle under the flowing stream. DO NOT allow the bottle to touch the faucet or water to splash up onto the faucet.
f. The sample bottle must be filled to the proper amount. The 120 mL bottle must be filled so the water is just above the fill line. The 150 mL bottle must be filled to a level between the two embossed lines (see photo below). **Too little or too much water will result in the sample being rejected and you will be required to recollect the sample.**
g. Quickly remove the container from the flowing stream. Replace the cap carefully and tightly.
h. Turn off the water. Replace any attachments that were removed from the faucet.

i. Wipe the bottle dry and **place label on the bottle.**
j. If your system chlorinates, a chlorine residual must be measured at the same time and location as the bacteriological sample. Community water systems (PWSID starts with the #1) must measure a total chlorine residual. Noncommunity water systems (PWSID starts with the #5) must measure a free chlorine residual. Report the chlorine level on Chain of Custody.
5. **Complete Chain-of-Custody form using indelible ink:**
a. Name and phone number of the Collector
b. Date and time collected (include a.m. or p.m.)
c. Chlorine residual (if required)
d. Put your signature on the Relinquished By line, including date and time. The date and time are when the sample is put in the return mailer and sealed. If samples pass hands prior to packaging, both parties must sign, date, and time. The first party would put down the date and time of the transfer, and the second party would put down the date and time the sample is packaged.
6. **Shipping and Handling**
a. Mail the sample as soon after collection as possible. Only collect and mail the sample on a Monday, Tuesday, or Wednesday.
b. Place the bottle and the Chain of Custody in the return mailer provided by the lab.
c. Bring the mailer to a courier that will guarantee overnight/express shipment to your designated laboratory.

TEST METHODS

All coliform tests done for compliance with the Safe Drinking Water Act must be performed by a laboratory approved for such testing by the Minnesota Department of Health (MDH). Three basic test methods are used to establish the presence of coliform bacteria. Selection of the test method is the responsibility of the laboratory and MDH. The laboratory must be certified by the state to perform the method used. The technicians doing the tests must also be certified.

MULTIPLE TUBE FERMENTATION



Multiple tube fermentation is very rarely used. The multiple tube fermentation or most probable number (MPN) test progresses through two steps, the presumptive and confirmed test. A final check may be done by use of the completed test. The presumptive test is not exclusive for coliform bacteria; some other bacteria present in soil or water may also produce a presumptive test, but the confirmed test is specific for coliform bacteria. The completed test, used for quality-control purposes, definitely establishes the presence of coliform bacteria. Bacteriological testing of most public water supplies stops after the confirmed test. This is the minimum test required of the positive samples.

The presumptive test is the first step of the MPN test. Samples are poured into each of five tubes containing a culture media and an inverted vial. The samples are incubated at 37° C (98.6° F) for 24 hours, checked and then incubated for another 24 hours, then checked again. If coliform bacteria are present, gas will be forming in the inverted vial within the 48-hour period. This indicates a presumptive positive sample. If no gas forms, the sample is considered negative.

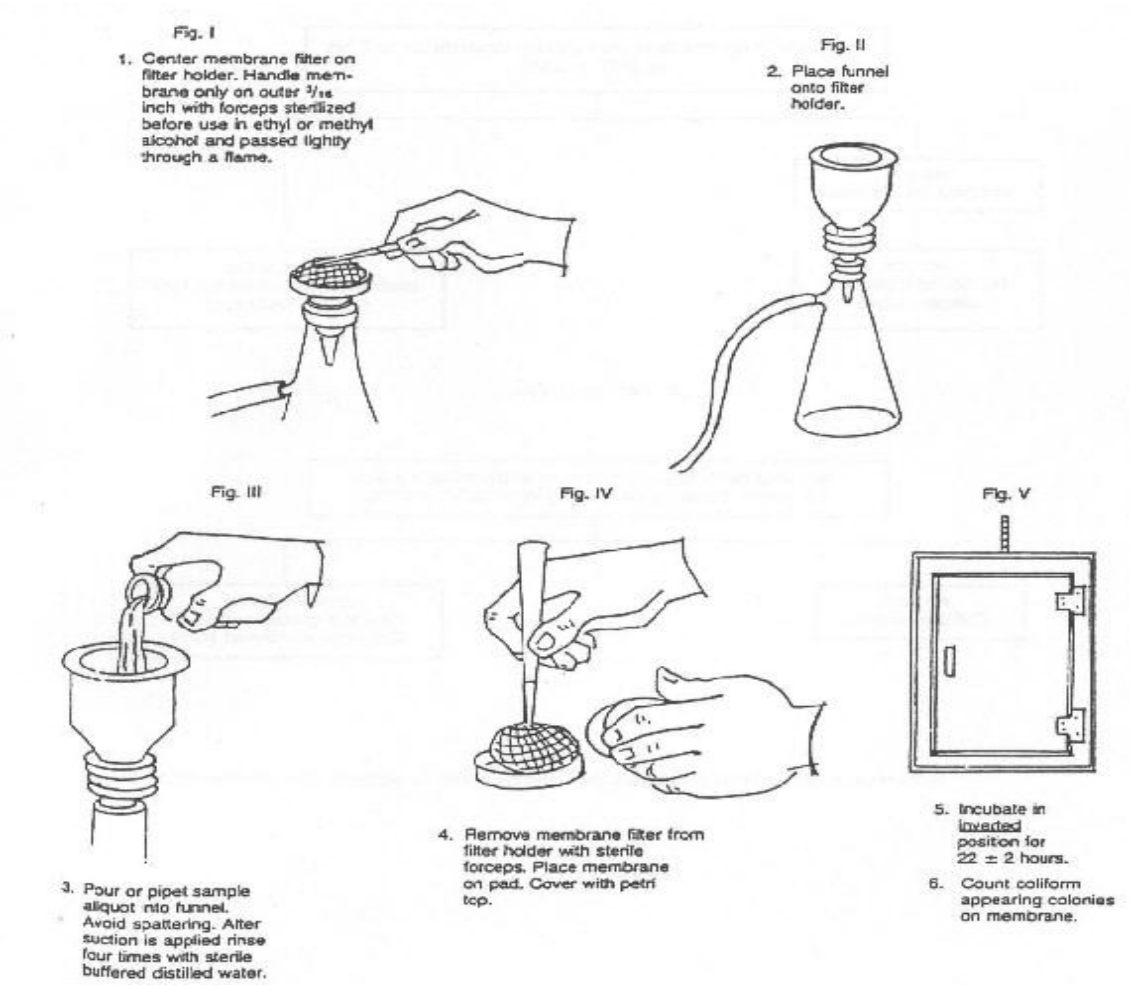
The confirmed test is more selective for coliform bacteria. Cultures from the positive samples in the presumptive test are transferred to brilliant green lactose bile broth tubes, also containing inverted vials, and incubated. If no gas is produced after 48 hours, the test is negative, meaning no coliform bacteria are present. If gas is produced, the test is positive, indicating the presence of coliform bacteria. From the number of positive samples found, the technician uses statistical (MPN) tables to determine the number of coliforms present in the original sample.

If further confirmation is needed, positive samples may be transferred to the completed test where the selection for coliform bacteria is even closer than for the confirmed test. The positive sample is transferred to a plate containing a special growth media and incubated for up to 24 hours. A second portion is placed in a lauryl tryptose broth and incubated for the same 18 to 24 hours. The completed test is positive if gas is formed in the lauryl tryptose broth and coliform bacteria are found on the plate. If no gas is formed, the test is considered negative.

MEMBRANE FILTER METHOD

Many private laboratories still use the membrane filter technique, but it is becoming less common. The membrane technique was, until recently, the most common method used to isolate coliform bacteria. A given size sample, generally 100 milliliters, is filtered through a membrane, small-pore filter, which is then incubated in contact with a selective culture agar at 37° C (98.6° F). A coliform bacteria colony will develop at each point on the membrane where a viable coliform was left on the membrane during filtration. After the incubation period of 24 hours, the number of colonies per plate is counted. They represent the actual number of coliforms that were present in the volume of samples filtered.

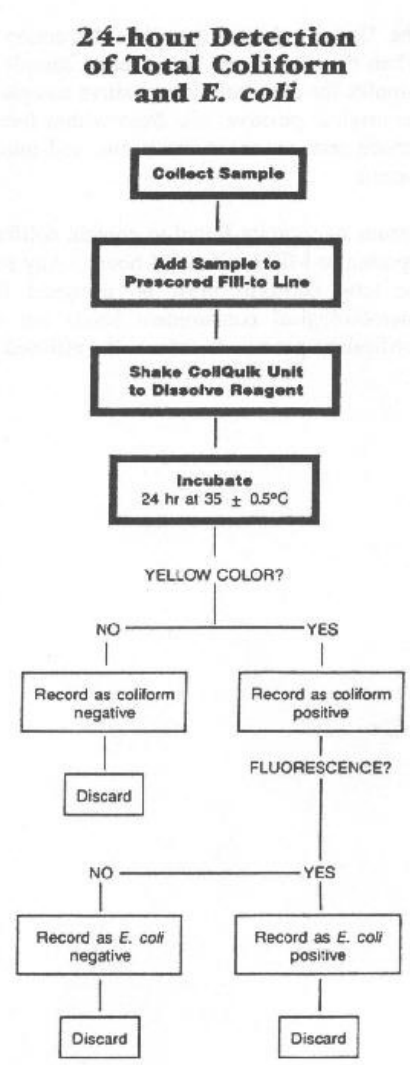
A typical coliform bacteria colony is pink to dark red with a distinctive green metallic sheen on the surface. All organisms that produce such colonies within 24 hours are considered members of the coliform group.



COLILERT TEST

The colilert test is probably the most widely used coliform detection method at this time and is a method accepted by the U. S. EPA for coliform testing. The colilert test is used for simultaneous detection and confirmation of both total coliforms and Eschericia coli (E. coli) fecal coliforms. As the colilert test is a presence/absence test, it does not indicate the extent of contamination. The colilert test method is just as accurate as the membrane filtration method and many believe it is more sensitive than the other methods.

The colilert reagent contains a formulation of salts, nitrogen, and carbon sources that are specific to total coliform. It contains specific indicator nutrients that create a yellow color when total coliforms are present and fluorescence when E. coil is present. The reagent is added to a 100-milliliter water sample in a sterile, nonfluorescent borosilicate glass container. The vessel is capped and shaken vigorously by repeated inversion to aid in mixing of the reagent. It is then incubated at 35° C for 24 hours. (An 18-hour preliminary test may be requested if there is reason to need test results more quickly.) After 24 hours, the technician compares the reaction vessels to the color in a comparator supplied with the test kit. If the inoculated reagent has a yellow color equal to or greater than the comparator, the presence of total coliform bacteria is confirmed.



The technician tests each reaction vessel for fluorescence by placing it five inches from an ultraviolet light in a dark environment.

POSITIVE TOTAL COLIFORM RESULT

When total coliform are found in a routine sample, the water supplier must collect at least four repeat samples for each coliform positive sample found. One repeat sample shall be from the site of the original positive, one from within five service connections upstream, one from within five service connections downstream, and one from another representative site on distribution system.

Results of samples found to contain coliform bacteria and results of all repeat samples must be reported to MDH within 24 hours. Any routine or repeat samples that are found to be positive for total coliform must be analyzed for fecal coliform or E. coli. When maximum microbiological contaminant levels are exceeded, the water supplier must use the proper notification process to inform the affected consumers.