

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; and 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
Cryptosporidiosis	Cryptosporidium parvum	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, i.e. total coliforms, indicates general contamination, while the presence of fecal coliforms 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. USEPA has set microbiological standards for drinking water, and public water suppliers that collect fewer than 40 samples per month are allowed one total coliform positive per month. Systems that collect 40 or more samples per month are allowed 5 percent total coliform positive results per month.

Fecal coliform provide stronger evidence of the possible presence of pathogens than do total coliform. This group indicates the presence of fecal matter, which could be of human or animal origin. One positive test does not prove that fecal contamination exists; and more tests must be taken. 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, however, involve errors in sampling. The current regulations require that if a sample is positive (shows the presence of coliforms), the water supplier must take four more 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.



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, and 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 and 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

Technique used in taking the sample is important. The following steps should be followed:

- Remove the aerator, if there is one, from the faucet. Some utilities will allow the tap to run for a short time and then flame the faucet with a torch. Flaming is not required but will assure that the outside of the tap is sterile. Caution is required as plastic faucets may melt if flamed. (The collector may also sterilize the tap with an alcohol pad.)
- Turn on the faucet and allow it to run for five to six minutes so that the sample drawn is from the distribution system, not the interior plumbing of the building. If the service is large, the tap should be run longer. The hot-water tap should not be used for sampling.
- After the lines have been flushed, open the container and hang on to the bottle cap, taking care to not touch the inside of the cap. Do not rinse the container and do not overfill.
- Place the sample container in the flow from the faucet without contacting the tap. Fill the bottle to the fill line, if provided. Leave space at the top of the container so that the laboratory can mix the sample before testing it. If the bottle is overfilled, take a new sample with a new container. The minimum sample required at the laboratory is 100 ml, and it is important not to flush out the preservative in the bottle.
- Seal the container as soon as it is filled and removed from the flow. If a sample bag is used, be sure it is sealed properly; these bags can leak easily.

Do the necessary paperwork, which includes filling in the PWS identification number, time, date, collector name, and location taken on the sample's label and on the reporting forms. The forms may require other information, such as pH, chlorine residual, and iron concentration.

If additional tests are required, don't use the same bottle used for total coliforms. Instead take a different sample from the same water faucet. Different types of tests require different types of bottles. Consult with laboratory prior to taking other types of samples to ensure that you use the correct sample bottle.

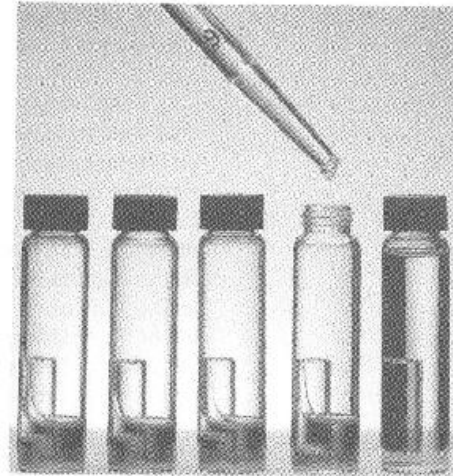
TEST METHODS

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. 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.

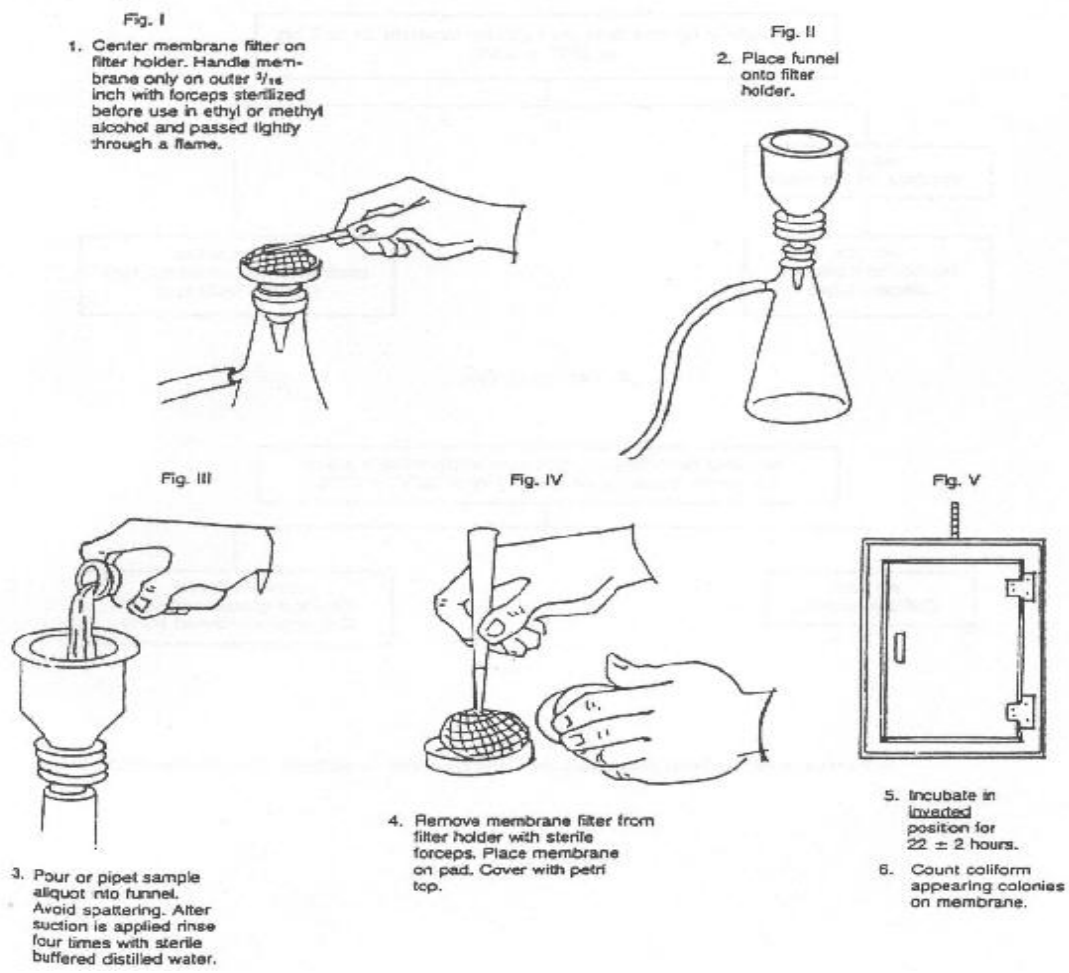
If further confirmation is needed, positive samples may be transferred to the completed test where the selection for coliform bacteria is even closer than for the confirmed test. The positive sample is transferred to a plate containing a special growth media and incubated for up to 24 hours. A second 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 *Escherichia 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. coli* is present. The reagent is added to a 100-milliliter water sample in a sterile, non-fluorescent 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.

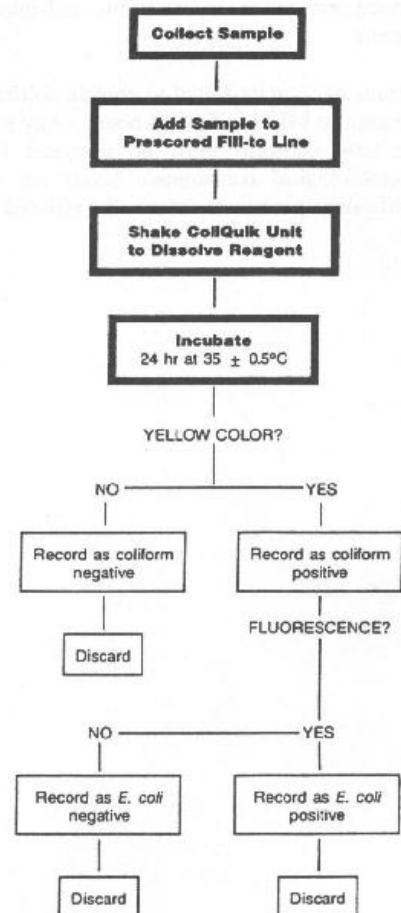
The technician tests each reaction vessel for fluorescence by placing it five inches from an ultraviolet light in a dark environment. If the

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.

24-hour Detection of Total Coliform and *E. coli*





Notes: