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Fecal Bacteria

What are fecal bacteria and why are they important?

Members of two bacteria groups, coliforms and fecal streptococci, are used as indicators of possible sewage contamination because they are commonly found in human and animal feces. Although they are generally not harmful themselves, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems. Therefore, their presence in streams suggests that pathogenic microorganisms might also be present and that swimming and eating shellfish might be a health risk. Since it is difficult, time-consuming, and expensive to test directly for the presence of a large variety of pathogens, water is usually tested for coliforms and fecal streptococci instead. Sources of fecal contamination to surface waters include wastewater treatment plants, on-site septic systems, domestic and wild animal manure, and storm runoff.

In addition to the possible health risk associated with the presence of elevated levels of fecal bacteria, they can also cause cloudy water, unpleasant odors, and an increased oxygen demand. (Refer to the section on dissolved oxygen.)

Indicator bacteria types and what they can tell you

The most commonly tested fecal bacteria indicators are total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci, and enterococci. All but *E. coli* are composed of a number of species of bacteria that share common characteristics such as shape, habitat, or behavior; *E. coli* is a single species in the fecal coliform group.

Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliform group can occur in human feces, but some can also be present in animal manure, soil, and submerged wood and in other places outside the human body. Thus, the usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found are fecal and human in origin. For recreational waters, total coliforms are no longer recommended as an indicator. For drinking water, total coliforms are still the standard test because their presence indicates contamination of a water supply by an outside source.

Fecal coliforms, a subset of total coliform bacteria, are more fecal-specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper mill wastes. Therefore, if these sources discharge to your stream, you might

wish to consider monitoring more fecal and human-specific bacteria. For recreational waters, this group was the primary bacteria indicator until relatively recently, when EPA began recommending *E. coli* and enterococci as better indicators of health risk from water contact. Fecal coliforms are still being used in many states as the indicator bacteria.

E. coli is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. EPA recommends E. coli as the best indicator of health risk from water contact in recreational waters; some states have changed their water quality standards and are monitoring accordingly.

Fecal streptococci generally occur in the digestive systems of humans and other warm-blooded animals. In the past, fecal streptococci were monitored together with fecal coliforms and a ratio of fecal coliforms to streptococci was calculated. This ratio was used to determine whether the contamination was of human or nonhuman origin. However, this is no longer recommended as a reliable test.

Enterococci are a subgroup within the fecal streptococcus group. Enterococci are distinguished by their ability to survive in salt water, and in this respect they more closely mimic many pathogens than do the other indicators. Enterococci are typically more human-specific than the larger fecal streptococcus group. EPA recommends enterococci as the best indicator of health risk in salt water used for recreation and as a useful indicator in fresh water as well.

Which Bacteria Should You Monitor?

Which bacteria you test for depends on what you want to know. Do you want to know whether swimming in your stream poses a health risk? Do you want to know whether your stream is meeting state water quality standards?

Studies conducted by EPA to determine the correlation between different bacterial indicators and the occurrence of digestive system illness at swimming beaches suggest that the best indicators of health risk from recreational water contact in fresh water are *E. coli* and enterococci. For salt water, enterococci are the best. Interestingly, fecal coliforms as a group were determined to be a poor indicator of the risk of digestive system illness. However, many states continue to use fecal coliforms as their primary health risk indicator.

If your state is still using total or fecal coliforms as the indicator bacteria and you want to know whether the water meets state water quality standards, you should monitor fecal coliforms. However, if you want to know the health risk from recreational water contact, the results of EPA studies suggest that you should consider switching to the *E. coli* or enterococci method for testing fresh water. In any case, it is best to consult with the water quality division of your state's environmental agency, especially if you expect them to use your data.

Sampling and equipment considerations

Bacteria can be difficult to sample and analyze, for many reasons. Natural bacteria levels in streams can vary significantly; bacteria conditions are strongly correlated with rainfall, and thus comparing wet and dry weather bacteria data can be a problem; many analytical methods have a low level of precision yet can be quite complex; and

absolutely sterile conditions are required to collect and handle samples.

The primary equipment decision to make when sampling for bacteria is what type and size of sample container you will use. Once you have made that decision, the same, straightforward collection procedure is used regardless of the type of bacteria being monitored. Collection procedures are described under "How to Collect Samples" below.

It is critical when monitoring bacteria that all containers and surfaces with which the sample will come into contact be sterile. Containers made of either some form of plastic or Pyrex glass are acceptable to EPA. However, if the containers are to be reused, they must be sterilized using heat and pressure. The containers can be sterilized by using an autoclave, which is a machine that sterilizes containers with pressurized steam. If using an autoclave, the container material must be able to withstand high temperatures and pressure. Plastic containers either high-density polyethylene or polypropylene might be preferable to glass from a practical standpoint because they will better withstand breakage. In any case, be sure to check the manufacturer's specifications to see whether the container can withstand 15 minutes in an autoclave at a temperature of 121°C without melting. (Extreme caution is advised when working with an autoclave.) Disposable, sterile, plastic Whirl-pak® bags are used by a number of programs. The size of the container will depend on the sample amount needed for the bacteria analysis method you choose and the amount needed for other analyses.

There are two basic methods for analyzing water samples for bacteria:

1. The membrane filtration method involves filtering several different-sized portions of the sample using filters with a standard diameter and pore size, placing each filter on a selective nutrient medium in a petri plate, incubating the plates at a specified temperature for a specified time period, and then counting the colonies that have grown on the filter. This method varies for different bacteria types (variations might include, for example, the nutrient medium type, the number and types of incubations, etc.).

2. The multiple-tube fermentation method involves adding specified quantities of the sample to tubes containing a nutrient broth, incubating the tubes at a specified temperature for a specified time period, and then looking for the development of gas and/or turbidity that the bacteria produce. The presence or absence of gas in each tube is used to calculate an index known as the Most Probable Number (MPN).

Given the complexity of the analysis procedures and the equipment required, field analysis of bacteria is not recommended. Bacteria can either be analyzed by the volunteer at a well-equipped lab or sent to a state-certified lab for analysis. If you send a bacteria sample to a private lab, make sure that it is certified by the state for bacteria analysis. Consider state water quality labs, university and college labs, private labs, wastewater treatment plant labs, and hospitals. You might need to pay these labs for analysis.

This manual does not address laboratory methods because several bacteria types are commonly monitored and the methods are different for each type. For more information on laboratory methods, refer to the references at the end of this section. If

you decide to analyze your samples in your own lab, be sure to carry out a quality assurance/quality control program. Specific procedures are recommended in the section below.

How to Collect Samples

The procedures for collecting and analyzing samples for bacteria consist of the following tasks:

TASK 1 Prepare sample containers

If factory-sealed, presterilized, disposable Whirl-pak® bags are used to sample, no preparation is needed. Any reused sample containers (and all glassware used in this procedure) must be rinsed and sterilized at 121 C for 1 5 minutes using an autoclave before being used again for sampling.

TASK 2 Prepare before leaving for the sampling site

Refer to section 2.3 - Safety Considerations for details on confirming sampling data and time, picking up equipment, reviewing safety considerations, and checking weather and directions. In addition, to sample for coliforms you sh ould check your equipment as follows:

- Whirl-pak® bags are factory-sealed and sterilized. Check to be sure that the seal has not been removed.
- Bottles should have tape over the cap or some seal or marking to indicate that they have been sterilized. If any of the sample bottles are not numbered, ask the lab coordinator how to number them. Unless sample container s are to be marked with the site number, do not number them yourself.

TASK 3 Collect the sample

Refer Task 2 in Chapter 5 - Water Quality Conditions for details on collecting a sample using screw-cap bottles or Whirl-pak® bags. Remember to wash your hands thoroughly after collecting samples suspected of containing fecal contamination. Also, be careful not to touch your eyes, ears, nose, or mouth until you've washed your hands.

Recommended field quality assurance/quality control procedures include:

- Field Blanks. These should be collected at 10 percent of your sample sites along with the regular samples. Sterile water in sterilized containers should be sent out with selected samplers. At a predetermined sample site, the sampler fills the usual sample container with this sterile water. This is labeled as a regular sample, but with a special notation (such as a "B") that indicates it is a field blank. It is then analyzed with the regular samples. Lab analysis should result in "0" bacteria counts for all blanks. Blanks are used to identify errors or contamination in sample collection and analysis.
- Internal Field Duplicates. These should be collected at 10 percent of your sampling sites along with the regular samples. A field duplicate is a duplicate

stream sample collected at the same time and at the same place either by the same sampler or by another sampler. This is labeled as a regular sample, but with a special notation (such as a "D") that indicates it is a duplicate. It is then analyzed with the regular samples. Lab analysis should result in comparable bacteria counts per 100 mL for duplicates and regular samples collected at the same site. Duplicates are used to estimate sampling and laboratory analysis precision.

- External Field Duplicates. An external field duplicate is a duplicate stream sample collected and processed by an independent (e.g., professional) sampler or team at the same place at the same time as regular stream samples. It is used to estimate sampling and laboratory analysis precision.

TASK 4 Return the field data sheets and the samples to the lab or drop-off point

Samples for bacteria must be analyzed within 6 hours of collection. Keep the samples on ice and take them to the lab or drop-off point as soon as possible.

TASK 5 Analyze the samples in the lab

This manual does not address laboratory analysis of water samples. Lab methods are described in the references below (APHA, 1992; River Watch Network, 1991; USEPA, 1985). However, the lab you work with should carry out the following recommended laboratory quality assurance/quality control procedures:

- Negative Plates result when the buffered rinse water (the water used to rinse down the sides of the filter funnel during filtration) has been filtered the same way as a sample. This is different from a field blank in that it contains reagents used in the rinse water. There should be no bacteria growth on the filter after incubation. It is used to detect laboratory bacteria contamination of the sample.

- Positive Plates result when water known to contain bacteria (such as wastewater treatment plant influent) is filtered the same way as a sample. There should be plenty of bacteria growth on the filter after incubation. Positive plates are used to detect procedural errors or the presence of contaminants in the laboratory analysis that might inhibit bacteria growth.

- Lab Replicates. A lab replicate is a sample that is split into subsamples at the lab. Each subsample is then filtered and analyzed. Lab replicates are used to obtain an optimal number of bacteria colonies on filters for counting purposes. Usually, subsamples of 100, 10, and 1 milliliter (mL) are filtered to obtain bacteria colonies on the filter that can be reliably and accurately counted (usually between 20 and 80 colonies). The plate with the count between 20 and 80 colonies is selected for reporting the results, and the count is converted to colonies per 100 mL.

- Knowns. A predetermined quantity of dehydrated bacteria is added to the reagent water, which should result in a known result, within an acceptable margin of error.

- Outside Lab Analysis of Duplicate Samples. Either internal or external field duplicates can be analyzed at an independent lab. The results should be comparable to those obtained by the project lab.

References

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Water Quality Sampling Field Data Sheet (PDF, 4.41 KB)

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