FECAL BACTERIA BEST MANAGEMENT PRACTICES (BMPs)

►Fecal Bacteria Sampling And Testing

OVERVIEW

It can be difficult to notice when fecal bacteria may be present in waterbodies or storm water runoff because the presence of clear sensory or visual indicators, such as foul odors or toilet paper, are not present. As a result, fecal bacteria contamination can go unnoticed for extended periods. Therefore, sampling and testing for fecal bacteria is imperative to determine if excessive fecal bacteria that could harm the ecosystem and human health is present.

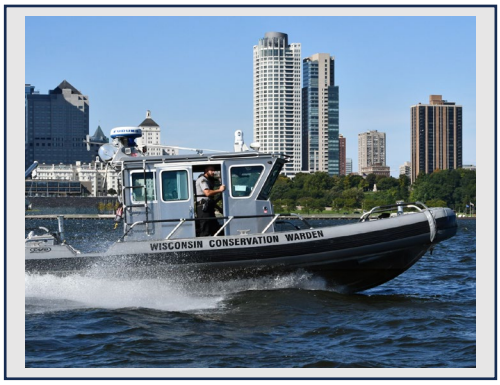

Photo Credit: Wisconsin DNR

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The U.S. EPA's recommended limit of E. coli is 235 CFU ("Colony Forming Units" or cells) per 100 mL for recreational waters to ensure waters are safe for recreational use (e.g., swimming). To determine if these levels are exceeded, there are many ways to test for fecal contamination in water, and the type of test used depends on your sampling needs. Below are some scenarios of when certain testing methods may be appropriate:

- Specific laboratory testing capabilities may be more applicable in a situation where the fecal contamination source origin is unknown. For example, genetic testing will indicate the source of fecal bacteria (e.g., human, bird, livestock, etc.) which is helpful to identify the source's origin and help identify practices to eliminate the fecal contamination.
- It is important to consider whether samples are taken under dry weather/low flow conditions or wet weather/high flow conditions.

FECAL BACTERIA INDICATORS

Total Coliforms

Bacteria within this group can be naturally found in the environment, but also include groups associated with animal waste and human sewage.

Fecal Coliforms

This is a group of bacteria found in human and animal waste. Though fecal coliforms can indicate risk to human health, fecal coliforms are a less specific indicator because the bacteria group is still too broad.

E. coli

This is a specific bacteria species naturally found in warm-blooded animals (e.g., humans, wildlife, etc.). Studies have found a link between the presence of *E. coli* and sewage contamination. Testing for E. coli is recommended by the EPA because it is the best indicator of other pathogens present in the water. The State of Wisconsin has historically used fecal coliform as an indicator for fecal bacteria contamination, however, in May 2020 the State adopted an *E. coli* criterion as a more specific fecal indicator in surface waters (Wis. Adm. Code NR 102; Geometric mean of 126 CFU/100mL). While this species indicates the presence of fecal matter, it is not able to distinguish between human and animal sources.

Enterococci

This is an alternative fecal bacteria species indicator naturally found in human and animal waste. Like E. coli, studies have identified a correlation between the presence of Enterococci and fecal contamination – however, in some cases, Enterococci have been found to persist in the environment outside of their host in freshwater systems. Due to this, the use of Enterococci is a commonly used indicator in marine coastal environments (e.g., beaches). Like E. coli, this test is not able to distinguish between human and animal sources.

Human Bacteroides

This is another fecal bacteria indicator used in genetic testing. More specifically, a human specific genetic marker of Bacteroides is often used to test if water samples contain fecal bacteria from human sources which are considered to have the greatest human health impact. A sample with high levels of this genetic marker could be a sign of illicit connections or sanitary sewer overflows.

SAMPLING PROCEDURES

Dry Weather Outfall Screening

This test is performed on outgoing storm water. Dry weather outfall screenings are done during dry conditions when there is no storm water coming from outfalls. Therefore, there should be no flow observed. However, if flow is present and there is a high loading of fecal bacteria, this can be indicative of point source pollution (e.g., illicit connections).

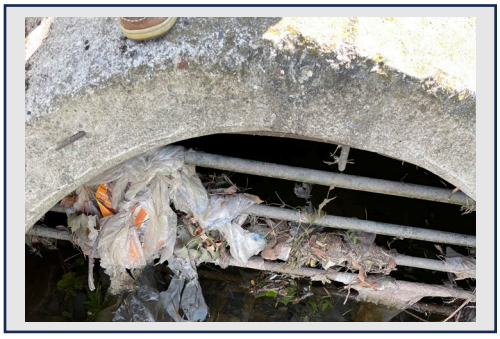

Photo Credit: Wisconsin DNR

Wet Weather Sampling

This sampling method for fecal bacteria happens immediately following a rain event. This test is useful in understanding fecal bacteria levels under medium or high-flow scenarios. This can be done by installing automated samplers that can measure flow and obtain a sample that can be analyzed for fecal bacteria or by taking a sample by hand at a storm water outfall. High loading of fecal bacteria under high flow conditions can be indicative of non-point source pollution (e.g., build-up, wash-off from a large parking lot).

COMMON TESTING METHODS

Below are some common field and lab testing methods for fecal bacteria along with a brief overview of sample procedures. For more information on example procedures and testing equipment, please see the Additional Resources section.

Quanti-Tray Method (*Quanti-Tray System – IDEXX US*)

This method involves adding water samples to a sealed tray, incubating and then counting wells based on color characteristics. This method is performed in a laboratory setting:

Summary Of Quanti-Tray Procedure

- Take a known volume of the water sample and add a reagent (e.g., Colilert.)
- Fill tray with water sample and seal the tray (e.g., Quanti-Tray sealer).
- Incubate for 24 hours typically, at 35 degrees Celsius.
- Color indicates the presence of bacteria; wells that have turned yellow indicate total coliform presence. Then place the tray under a UV light, and wells that fluoresce indicate *E. coli* presence.
- Quantify the number of bacteria using a Most Probable Number (MPN) table *IDEXX Quanti-Tray*/2000 MPN Table*

Advantages And Disadvantages

- Trays are relatively inexpensive; sealers and reagents are expensive.
- Good for primary screening, determining whether fecal bacteria are present and roughly how much.
- Relatively easy to quantify by counting the number of positive wells and using an MPN table, no colony counting required.
- Doesn't distinguish between human or animal origins.
- Needs to be done in lab, would not be practical with citizen science monitoring programs.

Plating Method

This method involves growing fecal bacteria colonies from water samples on growth media, and then counting the number of colonies present. This method can be done in the field or laboratory setting using filtration methods or testing kits like the 3M Petri film test; however, the use of an incubator is a necessary step.

Filtration Procedure (EPA Method 1103.1):

- Filter water sample through a sterile membrane filter. The size of the membrane filter for E. coli is typically a 0.45 µm pore size.

- Place the filter membrane on selective growth media plates (e.g., m-FC agar, m-TEC agar).
- Incubate plates for 24 hours typically, at 35 degrees Celsius.
- Count the number of colonies present.

Advantages And Disadvantages

- Materials are relatively inexpensive, but an incubator is needed for accurate measurements. This option is cheaper than Quanti-Tray Sealers and genetic testing equipment.
- Good for primary screening, determining whether bacteria are present and roughly how much.
- Counting and interpreting results is time intensive and requires technical skills, which may not be practical for watershed-scale monitoring.
- Not practical for community science monitoring programs.
- Doesn't distinguish between human or animal origins.
- If bacteria concentrations are high enough, results can be too numerous to count (TNTC), which is no longer quantitative.

3M Petrifilm Procedure (*3M™ Petrifilm™*):

- Add the water sample to the Petri film. This process can occur when out in the field or in the laboratory.
- Incubate sample for 24 hours.
- Count present colonies using 3M's guide (*Interpretation Guide*).

Advantages And Disadvantages

- Can be done in the field or the lab.
- Relatively easy to use.
- Materials are relatively inexpensive, but an incubator is needed for accurate results. This option is cheaper than Quanti-Tray Sealers and genetic testing equipment.
- Good for primary screening, determining whether bacteria are present and roughly how much.

- More practical for citizen-science monitoring programs as they don't need to operate expensive equipment or perform highly technical tasks.
- Could be more practical for watershed-scale monitoring programs.
- Doesn't distinguish between human or animal origins.
- If bacteria concentrations are high enough, results can be too numerous to count (TNTC), which is no longer quantitative.

Genetic Testing

This method involves testing for specific gene markers in the water sample. This method can be useful in identifying the concentrations of bacteria (e.g., E. coli) present in the sample or can be more specific in identifying the type of fecal bacteria source (e.g., dog, bird, human, etc.).

Summary Of Genetic Testing Procedure:

- Filter the water sample and extract the bacterial DNA that was captured on the membrane filter. DNA extraction kits are sold by many science research suppliers.
- Amplify the extracted DNA using a technique called Polymerase Chain Reaction (PCR):
    - Specific primers that target *E. coli* or the source of interest (e.g., human marker) are added to the extracted DNA sample as well as DNA Polymerase to stimulate DNA replication.
    - After PCR, gel electrophoresis is typically performed on the sample to verify the correct segment of DNA was amplified.
- Analyze amplified samples using quantitative PCR (qPCR), which will determine how much fecal bacteria was present in the water sample.

Advantages And Disadvantages

- Can identify the source of fecal bacteria depending on the marker used. Can help in identifying sources and targeting BMPs to address source(s).
- Good for areas that are known for excessive bacteria but don't know the source.
- Expensive as this requires specialized equipment.
- Performing the procedures and interpreting results requires technical training.
- Not practical with citizen-science monitoring programs.

ADDITIONAL RESOURCES

Sampling Procedures

- Dry Weather Screening and Catchment Investigation – UConn
- Bacteria Monitoring Training Manual – Milwaukee Riverkeeper
- Standard Operating Procedure, E. coli and Total Coliforms Using Quanti-tray – California Water Boards

Genetic Testing In Wisconsin

- **Public Environmental and Water Testing and Prices – Wisconsin State Lab of Hygiene**
- **McLellan Lab | UWM School of Freshwater Sciences**

Lab Testing Information And Equipment

- Quanti-Tray System – IDEXX US
- Interpretation Guide – 3M Petrafilm Test
- FastDNA™ Spin Kit for Soil DNA Extraction – MP Biomedicals
- Real-Time PCR Assays – Thermo Fisher Scientific
- StepOne™ Real-Time PCR System – Thermo Fisher Scientific
- Real-Time PCR Basics – Thermo Fisher Scientific

SOURCES

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Last Updated: October 2024