Milwaukee Riverkeeper Bacteria Monitoring Training Manual

Introduction

What is Bacteria & Why are We Concerned?

Bacteria represents a group of unicellular microorganisms that lack specialized structures called organelles. Bacteria has a bad association; while some bacteria are bad, many forms of bacteria are essential to life on our planet. For example, humans have bacteria in their stomachs which aids in digestion and a bacterium in soil, called rhizobium, fixes nitrogen playing an important role in plant growth.

However, some bacteria can have impacts on human health. Escherichia coli or *E. coli* lives in human's intestines and keeps our digestive tract healthy. However, when it can find its way into our water it can lead to health problems for those using the water to recreate. *E. coli* can cause abdominal cramping, vomiting and kidney failure.

E.coli As a Water Quality Indicator

E. coli lives in the human gut, making the presence of *E. coli* in a waterway almost always a sign of fecal matter contamination. This unique indicator of human waste contamination makes testing for *E. coli* the preferred method of monitoring bacterial contaminantion in waterways used for recreating. In 2012 the EPA set new criteria for monitoring of recreational water quality. **The EPA standard for *E. coli* is 235cfu/100mL.**

Our Monitoring Program

Milwaukee Riverkeeper is piloting their Bacteria Monitoring Program to begin to integrate bacteria monitoring into the successful Water Quality Monitoring program. Below are methods adapted for citizen science monitoring of bacteria in the Milwaukee River Basin.

When is Bacteria Monitored?

Bacteria is monitored on a monthly basis from May through October. Sampling after a rain event may be ideal if you would like to know if your site experiences runoff.

Sampling Procedure

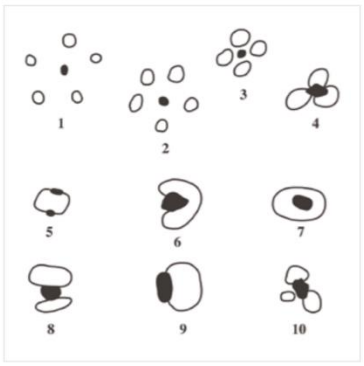
Equipment:

- 3M Petrifilm Plates
- Gloves
- 1 mL disposable pipettes
- Chicken Incubator
- Marker for labeling
- Data sheet
- Bleach
- Magnifier Lens
- Small collection bottle
- Waders

Procedure

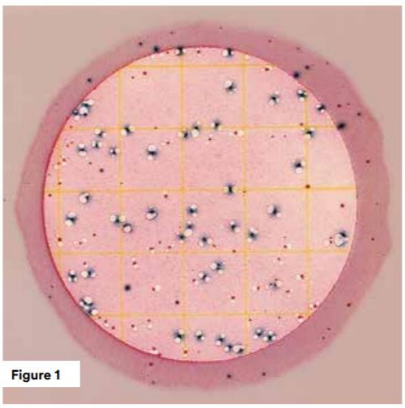
There are two different methods for sample collection. Samples can be gathered, stored on ice and processed at home or the sample can be processed on-site. Whichever method you decide to do, make sure you have the appropriate equipment. The process at home method will require a cooler and ice as additional materials.

1. Put on nitrile gloves
2. Using a marker, label the plate near the bottom with the site name and station ID number if available.
3. Enter your site from downstream heading upstream, choose an area with moving water that is at least 8 inches deep.
4. **Collection:** Triple rinse the collection bottle in the waterway prior to collection. Fill the bottle with a small amount of water, only 1 mL per plate is needed.
5. There are two inoculation methods, on-site or take home. For on-site inoculation, skip to step 6. If you are unable to inoculate the sample in the field. Cap the sample bottle and put it on ice immediately to be taken home to process at a later time.
6. **Inoculation**: Go to the river bank (if in the field) or pull your sample from the cooler if you are at home. Have a 3M Petrifilm plate ready. This step can be difficult; you may need to try it more than once.
    a. Using a 1 mL graduated pipette collect a 1 mL sample from your collection bottle. Lift the flap of the 3M Plate, drop 1 mL of the water sample on the middle part of the red circle. Begin closing the flap of the plate, gently tip the plate from side to side trying to cover the entirety of the circle.
    b. Once the circle is fully covered with water, the plate has been inoculated. If in the field, store the plate in the index card box to avoid it being damaged during travel. Bring your samples home to be incubated.
7. **Incubation**: Plug in the incubator, place the plate(s) into the incubator. Plates need to be incubated for 24 hours (+/- 2 hours) at 95 degrees Fahrenheit. Incubators should have a thermometer in them to keep track of temperature. Twist the metal handle to increase or decrease the incubator temperature as needed.
8. **Processing:** After the 3M plates have incubated for 24 hours you can begin to process the samples. Using the magnifier and a light source you can begin to count the number of colonies. We have found putting a source of light (cell phone flashlight) face up underneath the lid of the incubator and placing the 3M plate on top of the window is a good method for viewing the plate. This allows the light to shine up through the incubator as a nice source of light. Use the magnifier to count the number of colonies.



Bubbles

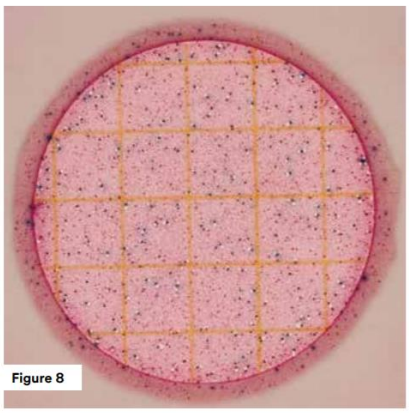
The illustrations above show examples of various bubble patterns associated with gas producing colonies. All should be enumerated.



***E. coli* count = 49 (blue colonies with gas)**

Total coliform count = 87 (red and blue colonies with gas)

Too Numerous to Count (TNTC)



Total coliform count = TNTC

Figure 1: Processing images: Bubble variation (left) E. coli/Total Coliform count (middle), Too Numerous to Count TNTC (right)

9. Blue colonies are *E. coli* and red colonies are total coliform. Only count colonies that are circler and have at least one bubble associated with the colony. See the Figure 1 of the various bubble patterns.
10. Count only the colonies that are within the circle. Any outliers are not to be included in the count. If there is an overgrowth of total coliform that makes it impossible to count, interpret as Too numerous to count or TNTC.
11. Complete the Bacteria Monitoring Data Sheet with count results and weather conditions, both recent rain events and current conditions.
12. Our procedure counts the number of colonies per one mL of water, to reference this against the EPA standard of **235cfu/100mL** multiple the number of *E. coli* colonies found times 100. This number will be the measurement of 100mL.
13. **Disposal**: Using the provided bleach, lift the plate flap and pipette a few drops of bleach to kill bacteria. Place the plate(s) into a ziplock bag, seal and dispose of in the trash.