

Citizens Monitoring Bacteria Sampling Plan

*** Depending on your location, you may need to sample on Monday, Tuesday, or Wednesday to get samples shipped overnight to the lab in time for them to complete the tests. ***

Before You Go Out to Sample

- Take 3 bottles of Easygel per each site out of freezer to thaw – if rapid thawing is required, they may be rinsed in warm water.
- Take 3M Petrifilm out of the fridge – 3 for each site.
- Turn on incubator – be sure the lid is tight and that it's the correct temp. (35°C) Fill appropriate channels in plastic tray with distilled water and set in bottom of incubator. Place wire tray on top.

Take to the Sampling Site

- Soap, antibacterial lotion or wipes
- plastic gloves
- waders
- cooler with ice
- Sharpie (to label bottles)
- Shipping containers/ice packs and forms
- Sterile collection containers (one per site)
- Sterile lab sample bottles (one per site)
- 2-3 data sheets (one per site) on clipboard
- 1 or 2 thermometers
- transparency tube
- sampling device with rope (if sampling from bridge)

At the Site

1. hang thermometer where it is not in direct wind or sunlight (for air temperature reading) – may take about 5 minutes to stabilize
2. complete top of data sheet, stream flow stage, and stream assessment comments
3. take water temperature (hold approximately 2 minutes in main stream flow) – record on data sheet
4. Rinse sterile collection bottle (500mL bottle) three times with sample water using proper sample collection technique – lower in upside down position to a depth of 3-5 inches below the water's surface (or approximately up to your wrist), fill at an angle facing upstream – be sure your hand and or fingers are not in front of the mouth of the bottle
 - If sampling from a bridge – rinse sampling device with stream water 3 times, then collect a sample and rinse the collection bottle three times – then fill collection bottle (be sure the bucket and rope do not come into contact with the ground during this process)
4. After rinsing the bottle 3 times, collect sample and top with lid after removing from stream – place collection bottle in cooler with ice for transporting
 - If shipping samples to lab before returning home/office, SHAKE COLLECTION BOTTLE TO MIX THE SAMPLE, then fill the lab sample bottle (100 mL) to its shoulder from the collection bottle (DO NOT rinse sample bottle 3 times; it may be filled with a preservative) – also put this bottle in cooler on ice.
5. record air temperature reading on data sheet
6. take transparency reading and record on data sheet
7. Wash hands when finished

Tips for Preparing/Plating the Samples

1. prepare table by cleaning with bleach and/or isopropyl alcohol
2. wash hands thoroughly with soap
3. Items to have at home/office “lab” station

- paper towels or Kimwipes
- isopropyl alcohol
- distilled water
- rinse/waste container
- Sharpie or permanent marker
- gloves

Number needed:	2 sites	3 sites
Collection & sample bottles	4	6
3M PetriFilm	6	9
Easygel bottles	6	9
Easygel petri dishes	6	9
Pipettes for sample transfer	2	3

4. Set up stations for each site (1 – 2 – 3)
 - You have one collection bottle and one lab sample bottle **per site**
 - You have 3 Petrifilm, 3 Easygel bottles, 3 Easygel petri dishes, and 1 pipette **per site**.
 - Label Petrifilm and Easygel bottles with site #s, label bottom of petri dishes with site # and volume (mL) of sample to be used.
5. ALWAYS SHAKE SAMPLE BOTTLE BEFORE DRAWING A SAMPLE WITH A PIPETTE!
6. Starting with site #1, add an appropriate volume of sample water (using a sterile pipette and drawing from the collection bottle) to the three duplicate Petrifilm and Easygel bottles. You will always use 1mL for the Petrifilm. You can choose between 0.5 mL up to 5 mL for the Easygel bottles. (Note: you can use the same pipette to transfer the sample water from site #1 to each of the appropriate tests if you use sterile technique.)
7. Complete the Petrifilm test by using the spreader
8. Complete the Easygel tests by inverting the bottles, pouring and swirling the plates

Complete steps 5-8 for sites #2 - #3.

Incubation (Remember to write down what time incubation begins!)

- Place plated samples in incubator: Easygel petri dish (upside down) and 3M Petrifilm (right side up) – three per site. Remember: Easygel needs to sit for at least 45 minutes to gel before placed in incubator upside down.
- After 24 hours, count E.coli colonies on both Petrifilm and Easygel plates
- After 48 hours, count E.coli colonies on both Petrifilm and Easygel plates

Which items need to be sterile?

- 2 bottles from the laboratory: 500mL sample collection bottle, 100mL lab sample bottle
- pipettes

Don't forget to **take photos** (or have someone take photos of you) at your site, and performing the methods – these are needed for our training manual and website! They can be electronic or hard copy.