Chapter 2

BACTERIAL MONITORING

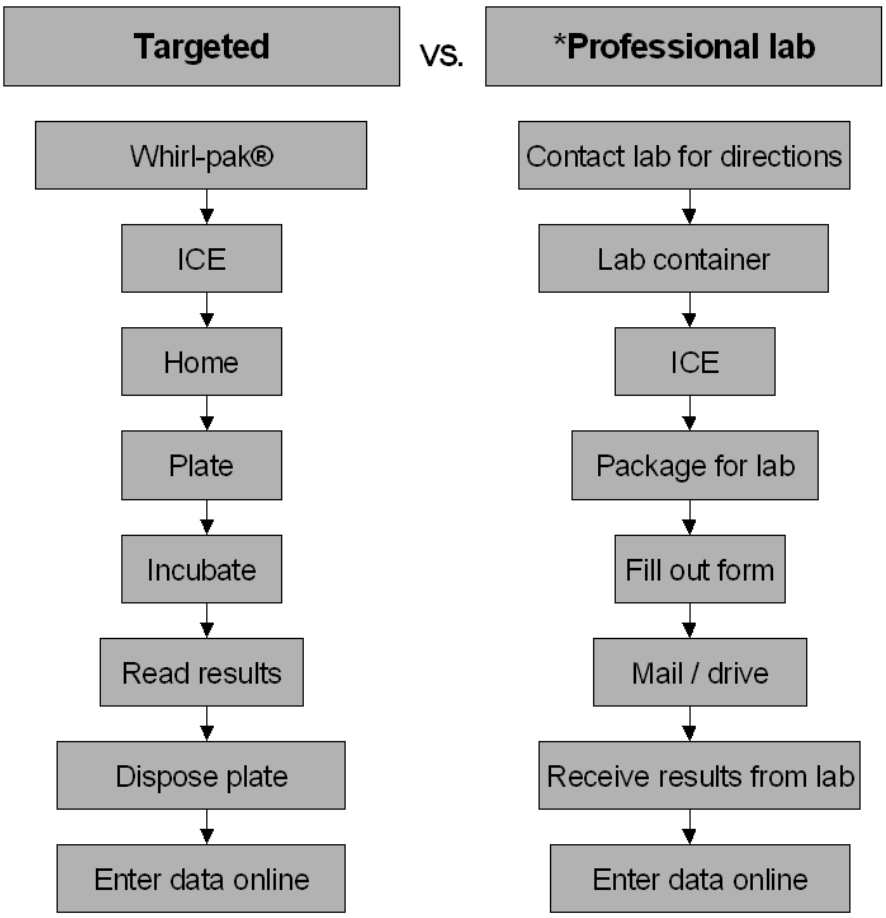
- When to Sample
- Choosing Your Sampling Method
- Equipment Needed for Bacterial Monitoring
- Directions for Bacteria Monitoring Using 3M Petrifilm™
- Reading the Results
- Getting “High” Bacteria Counts
- Source Tracking

When to Sample

Georgia Adopt-A-Stream recommends that sampling for *E. coli* should be done at least monthly during dry weather conditions. You should also be consistent with the time of day you sample. These factors help in the comparison of your data over time. The number of times that you’ll need to sample varies and depends on your goals. The more you sample, however, the better information you will have when interpreting your data.

Choosing Your Sampling Method

Volunteers have the option of processing their samples at home using 3M™ Petrifilm™ plates and an incubator or by packaging and shipping samples to a professional lab. Many studies have shown that the 3M™ Petrifilm™ method is as effective as the professional lab methods and is more practical and cost efficient for the volunteer monitor. If you need professional quality lab data, please refer to page 36 for more information about Adopt-A-Stream’s partnership with UGA Cooperative Extension Service’s water quality lab. Shown below is a flow chart that provides a general guide to process water samples taken from the field.



For more information comparing these methods, see The Volunteer Monitor's Bacteria Methods Comparison Study at http://water.epa.gov/type/rsl/monitoring/upload/2006_03_20_monitoring_volunteer_newsletter_volmon18no1.pdf.

Equipment Needed for Bacterial Monitoring

After a thorough review of the various testing methods, Adopt-A-Stream has developed a list of equipment that is required for *E. coli* monitoring using 3M Petrifilm™ plates. This method requires an incubator that has to be maintained at a specific temperature. For equipment specifications and ordering information, please see Appendix A.
To collect a sample in the field, you will need:

- Bacterial data form
- Rubber boots, waders or old tennis shoes
- Bucket with rope or grab sample pole (if sampling from a bridge)
- Whirl-pak® bags (labeled with site and collection information)
- Latex or vinyl gloves
- Permanant marker
- Disinfected cooler with ice

- First aid kit
- 'Who to Call List'
- Trash bag to pick up litter

To plate and incubate samples at home, you will need:

- Bacterial data form
- Cup to hold Whirl-pak® bags
- 3M™ Petrifilm™ *E.coli* plates
- 1ml fixed-volume pipettor & sterile tips
- Clean space for sample processing with good lighting
- Incubator
- Digital thermometer
- Permanent marker
- Latex or vinyl gloves
- Safety glasses
- 10% Bleach solution, OR Lysol spray/disinfectant, AND watertight bag for sample disposal

Directions for Bacteria Monitoring Using 3M Petrifilm™

Part 1: Preparing the blank/control sample

For each sampling event (i.e. a day of sampling up to 10 sites), the volunteer shall fill a Whirl-pak® bag with distilled water at the first sample site to serve as the blank/control. Having a field blank when you sample is necessary to serve as a control. A control will ensure that you are practicing a sterile technique that prevents contamination. If you are sampling at more than one site, prepare one blank for every 10 sites. The blank is then plated and analyzed with the stream samples in the lab. Lab analysis of the blank should result in a zero reading for bacteria. If it is contaminated, you will need to discard all samples; no data can be submitted for these samples. Prior to collecting the control sample, label the Whirl-pak® bag with the stream name, site number, date and time collected, and the sample collector.

1. While in the field, correctly label 1 Whirl-pak® bag with a permanent marker for the blank/control.
2. Put on latex gloves and remove the perforated seal from the top of the Whirl-pak® bag.
   IMPORTANT! Do not touch the inside of the Whirl-pak® as this will contaminate your sample and alter the results.
3. Use the two small white tabs to pull open the bag.
4. Fill the Whirl-pak® bag 2/3 full with distilled water.

5. Grab the ends of the twist ties and “whirl” or spin the bag tight. Cross the twist ties to close the bag.
6. Make sure the bag is closed securely by inverting the bag to test the seal (no water leaks out).
7. Immediately place the Whirl-pak® bag into a properly disinfected cooler with ice and store there throughout your sampling event.

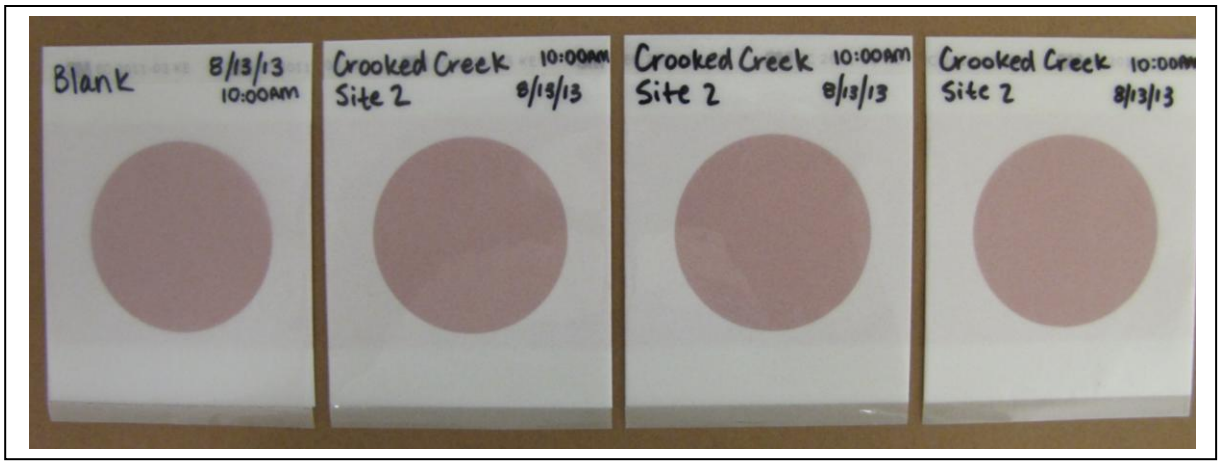
Part 2: Collecting site samples in the field

1. Correctly label new Whirl-pak® bag with a permanent marker for the sample/site information.
2. Put on latex gloves and remove the perforated seal from the top of the Whirl-pak® bag.
   IMPORTANT! Do not touch the inside of the Whirl-pak® as this will contaminate your sample and could alter the results.
3. Use the two small white tabs to open the bag.
4. While holding the yellow twist ties place the bag in the water at mid-stream, mid-depth or in a well-mixed area and allow the water to flow into the bag. Fill the bag with water up to 2/3 full.
   *Remember to collect the water sample (at least wrist deep) upstream of where you are standing. If sampling from a bridge, use a rope tied to a small disinfected bucket to grab the sample.
5. Grab the ends of the twist ties and “whirl” or spin the bag until tight. Cross the twist ties to close the bag.
6. Make sure the bag is closed securely by inverting the bag to test the seal (no water leaks out).
7. Immediately place the Whirl-pak® bag into a cooler with ice.
8. Optimal holding time for samples on ice or refrigeration is less than 6 hours but no more than 24 hours.
9. Properly dispose of gloves.

Part 3: Plating your samples

Note: Turn incubator on before plating to ensure it will reach the correct incubation temperature. Process the blank/control sample first (use 1 plate) and then follow with the stream sample (use 3 plates). Use 1 pipette tip per sample bag.

1. Clean working area with disinfectant spray and let dry.
2. Put on latex gloves. NOTE* You should always wear these when handling the plates (even when going to read them).
3. Correctly label plates (1 for the blank & 3 for the site sample), and lay them on a clean, flat surface. Plates should indicate stream name, site number and the incubation start time and date. See below figure for examples of how to label plates.



4. Gently shake Whirl-pak® bag to ensure an even mix of sample.
5. Place the Whirl-pak® bag in a cup to keep from spilling and open the bag using the white tabs.
   IMPORTANT! Do not touch the inside of the Whirl-pak® as this will contaminate your sample and could alter the results.
6. Carefully remove pipette tip from sterile container. Don't touch the pipette tip inside of the sterile container and practice caution to ensure that the tip is not contaminated thereafter.
6. Pipette 1 ml of the sample using the fixed-volume pipettor.
7. Lift the top film of the Petrifilm™ plate and dispense 1 ml of sample on the center of the circular plate.
8. Slowly roll the top film down onto the sample until the plate is completely covered to prevent trapping air bubbles. Do not touch the center of the petrifilm plate.
9. If necessary, distribute the sample evenly by using the 3M® spreader or slightly tilting the Petrifilm™ plate back and forth. Tilting too much will cause the sample to pour out of the plate.
10. Leave plate undisturbed for one minute to allow the gel to solidify and then place in the incubator.
11. Repeat: Plate two more samples for a total of three plates per sample site.

Part 4: Incubating

1. Plan to turn on the incubator prior to plating to ensure it will be ready. Place the incubator lid on top.
2. Insert the thermometer into the incubator.
3. Once the incubator is at 35°C ± 1°C, place the processed Petrifilm plates in the incubator and reset the thermometer.
4. Incubate plates in a horizontal position, with the top film side up, in stacks of up to 20 plates. **Incubate for 24 ± 1 hour at 35 ± 1 degrees Celsius.**

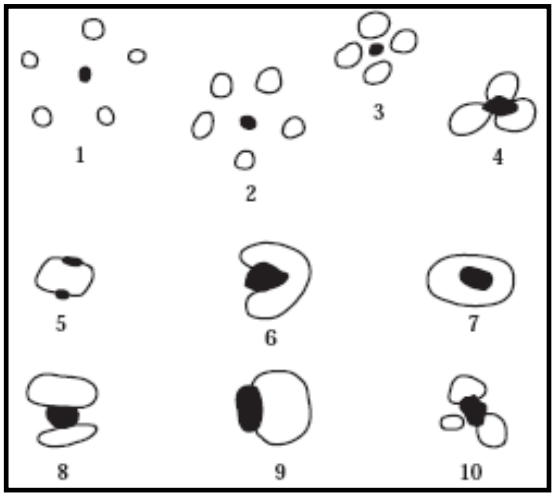
5. After 24 hours, remove plates (with gloves on) and count *E.coli* colonies.
6. Record the minimum and maximum temperatures that are displayed on the thermometer after incubating, as well as the time in/out of the incubator.
7. Record all data on the Bacterial Data Form.
8. Dispose of plates by spraying them with an appropriate disinfectant and placing in a sealed zip lock bag, discarding in the trash.

Part 5: Cleanup & Disinfect

Properly disinfect your lab space, incubator and cooler using 10% bleach disinfectant.

Reading the Results

When reading Petrifulm plates, ***E. coli*** colonies appear blue to red-blue and are closely associated with entrapped gas. **General coliform** colonies appear bright red and closely associated (approximately one colony diameter) with entrapped gas. Remember that we are only concerned with counting the *E. coli* colonies in the medium, and we do not count colonies that appear on the foam barrier of the plate. Gas bubble patterns associated with gas producing colonies are shown on the right. **Only count blue to red-blue colonies that have a gas bubble!**



Bacteria growths on plates are enumerated using a standard unit. The standard reporting unit is the number of **c**olony **f**orming **u**nits per 100 milliliters of water sample (cfu/100ml). Each Petrifilm plate holds 1mL of sample.

Exception: You might encounter a plate with colonies that are too numerous to count (TNTC) and that have one or more of the following characteristics: 150 *E. coli* colonies or more, many gas bubbles, and deepening of the gel color from red to purple-blue. High concentrations of *E. coli* will cause the entire growth area to turn a deep purple-blue color. The plate will be filled with colonies so much so that barely any empty space is present. If this happens, and the plate also contains blue to red-blue colonies, count them as presumptive *E. coli* whether or not gas bubbles are present.

To determine the number of colony forming units (cfu) per 100 ml of water sample, the following steps should be taken:

STEP I. Count the number of *E.coli* colonies on all three of your plates and add them together.	⇨	Let's assume you counted 6, 7, and 8 colonies = 21 colonies
STEP II. Find the average number of colonies. Take the total number of colonies and divide them by the number of plates used.	⇨	21 colonies / 3 plates = 7
STEP III. Now, multiply the average number of colonies by 100. You have now determined the number of colony forming units per 100 ml of sample.	⇨	7 x 100 = **FINAL COUNT** **700 cfu/100 ml**

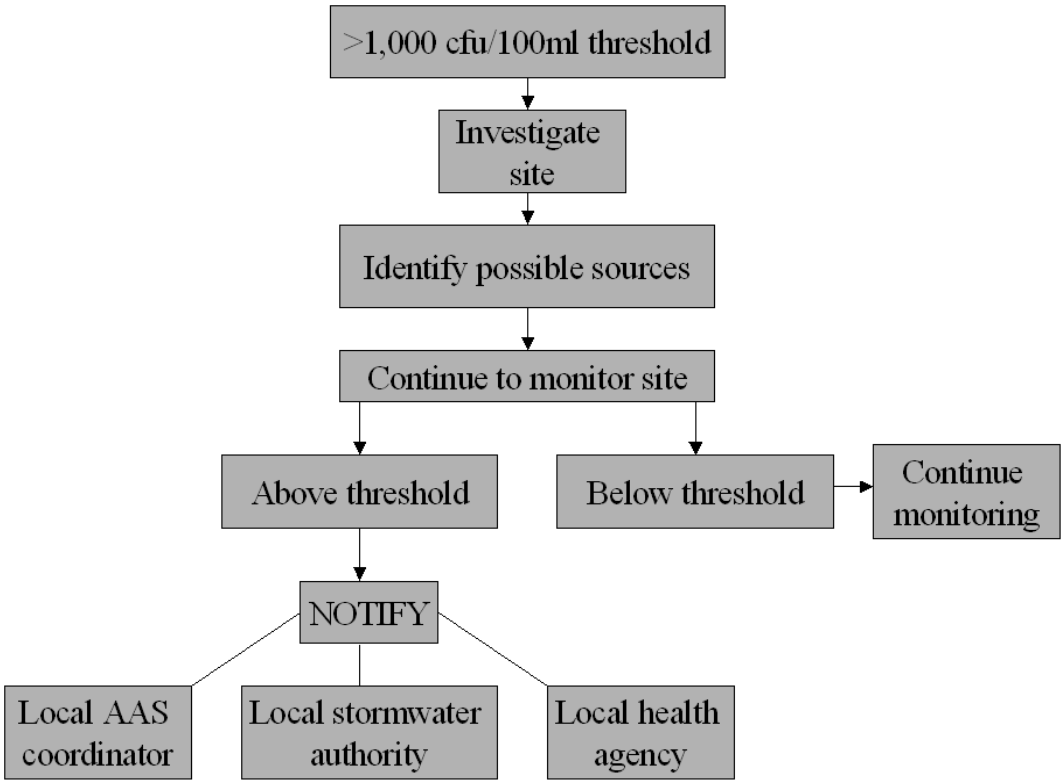
Getting "High" Bacteria Counts

Georgia Adopt-A-Stream recommends that *E. coli* sampling be done monthly during normal flow conditions. It is common to find high bacteria counts in urban areas. *E. coli* counts (cfu/100ml) that exceed 235 cfu/100 ml are considered "high" and should be closely monitored, but when counts exceed the 1000 cfu/100 ml threshold, they warrant special action. A count of 235 cfu/100 ml correlates to 8 incidents of 1000 people getting sick, but a count of 1000 cfu/100 ml correlates to about 14 incidents of 1000 people getting sick.

If you find a "high" bacterial count, it may be a one-time event or occurrence. This information is useful, but before taking further action, you should return to the site to take more samples. When you return, pay careful attention to anything out of the ordinary at the site. Look for the presence of animals and be alert for any unusual odors. Walk the banks again to look for obvious sources of pollution (see Chapter 2), and note past and current weather conditions. Continue to sample and contact your local health agency if numbers remain high. Be sure to wear gloves while sampling and wash your hands carefully afterwards.

If you continue to find counts above the 1000 cfu/100 ml threshold, work through your local Adopt-A-Stream coordinator to find the cause. You may also wish to alert your local watershed group or local agency about your monitoring efforts and the results so far. They may be able to work with you on determining the possible sources of *E. coli* pollution.

Detected High *E. coli* Counts: Follow These Steps



Using guidance provided by the US EPA, states have developed ambient standards for fecal coliform bacteria and/or *E. coli*. Compliance is often based on the average mean or the geometric mean of three or more samples taken during the same sampling event at representative locations within a defined sampling area. Detailed tables containing state fecal coliform standards and US EPA *E.coli* standards can be found in Chapter 1.

Source Tracking

One method for determining sources of *E. coli* is called Bacterial Source Tracking. Bacterial Source Tracking (BST) is a collective group of new methodologies being developed to determine sources of fecal pollution in environmental samples. Sources of fecal pollution include domestic pets, cows, deer, geese, hogs, other wild animals and humans.

If used successfully, BST methodologies have the potential to turn nonpoint (diffuse) sources into point sources. Current BST research is being driven by the recent implementation of the Total Maximum Daily Load (TMDL) concept by EPA. BST methods represent the best tools available for determining sources of fecal pollution in water and should be an integral part of any project that involves TMDL development for fecal coliforms. BST methods can also be used in the design and implementation of Best Management Practices (BMP) to reduce fecal loading in water.

Currently, both molecular (genotype) and biochemical (phenotype) BST methods are under development. DNA finger printing has received the greatest publicity, but numerous methods show potential. Most researchers believe that some combination of BST methods will be needed to provide the most accurate and reliable source identification answers. It is doubtful that any one BST method will emerge as the “best” method for all situations.

While this is not a procedure that the volunteers will be conducting, it is a procedure to be aware of, and a possible step that state agencies might take. At this point, it is still an emerging and costly technology, but is being incorporated more in government agencies.