Department of Natural Resources
Environmental Protection Division
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Georgia
Adopt-A-Stream
Volunteer Water Quality Monitoring

BACTERIAL MONITORING MANUAL

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Georgia Adopt-A-Stream

Georgia Department of Natural Resources

Environmental Protection Division

2 Martin Luther King Jr. Drive SE

Suite 1452, East Tower

Atlanta, GA 30334

AdoptAStream.Georgia.gov

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Special Contributions:

Environmental Protection Division, University of Georgia Cooperative Extension Service, Cobb County, Jones Ecological Research Center

Writers/Editors:

Georgia Adopt-A-Stream Staff

Some of the material in this manual was taken from the following documents:

Citizens Monitoring Bacteria: A Training Manual for Monitoring

Citizens Monitoring Bacteria manual, a product of the CSREES Great Lakes Regional Partnership

BACTERIAL MONITORING

Introduction

Welcome to the Georgia Adopt-A-Stream *Bacterial Monitoring* manual. This document provides background and directions for the program's QA/QC bacterial monitoring protocols which **help to protect water quality and streams** for the following reasons:

- Regularly monitoring a stream provides baseline information about its health
- Long-term trends as well as immediate changes in water quality can be documented
- Monitoring for bacteria provides information about water quality parameters that are important for the health of aquatic life

Bacterial testing allows volunteers to assess potential health risks due to bacterial contamination of surface waters.

Bacterial monitoring should be conducted regularly – at least once a month at the same time and the same location. Regular monitoring ensures comparison of data over a long period of time. Water quality and environmental conditions can change throughout the day, so monitoring at approximately the same time of day is important. Also, collecting bacterial samples after or during a rain event may produce very different results compared to regular flow conditions. Therefore, it is very important to record weather conditions. If conditions are unsafe for any reason, including high water or slippery rocks, **DO NOT SAMPLE!**

What are Bacteria?

Bacteria are single celled, living microscopic organisms. Under favorable conditions, bacteria can reproduce rapidly and can form colonies that are visible without magnification. Bacteria can utilize a large variety of habitats and can survive and adapt to almost all conditions on Earth. Their success and adaptability have allowed them to become the most numerous life forms on the planet. Most bacteria are beneficial and responsible for important environmental processes like decomposition, nutrient cycling, and the breakdown of environmental toxins. Some bacteria, however, are pathogenic (disease causing) and result in human health problems.

Coliform bacteria are members of the Enterobacteriaceae family. While some coliform bacteria are naturally found in soil, the type of coliform bacteria that lives in the intestinal

tract of warm-blooded animals originating from waste is called fecal coliform bacteria. *Escherichia coli (E. coli)* is a subgroup of fecal coliform bacteria. Within the *E. coli* species, there are numerous different strains, some of which are pathogenic. The presence of these naturally occurring organisms is not generally a cause for alarm, unless they appear at levels that exceed the US Environmental Protection Agency's (EPA) recommended limits.

What are Indicator Bacteria?

An indicator organism is one that signals certain conditions exist in an environment. The presence of fecal coliform bacteria indicates the possible presence of pathogens. Trying to detect disease-causing bacteria and other pathogens in water require considerable training, time, and financial commitment. The US EPA recommends using *E. coli* as indicator organisms of fecal contamination because:

- Their association with warm-blooded animal waste
- They generally live longer than pathogens
- They are typically found in great numbers
- Culturing *E. coli* is less risky than culturing pathogens in a lab

The presence of *E. coli* does not necessarily mean that pathogens are present but rather indicates a potential risk to human health. Monitoring for these indicator organisms is a simple and economically friendly way for citizens or professionals to assess health risks due to bacterial contamination of surface waters. If bacterial contamination of surface water is found, other disease-causing organisms may be present in this water and potentially pose a threat to human health.

How do Bacteria Enter Waterways?

E. coli in waterways may originate from the intestinal tracts of warm-blooded animals like dogs, cats, livestock, or humans. Human sources include failing septic tanks, sewage leaks, wastewater treatment plants, sewer overflows, land application of biosolids, boat discharges, and urban storm water runoff. In urban watersheds, fecal indicator bacteria are significantly correlated with human density. Possible animal sources of fecal coliform bacteria include cattle in streams, land application of animal waste, dairy operations, poultry operations, horse farms, pet waste from parks, lawns, streets, and wildlife such as geese, pigeons, ducks, deer, and raccoons.

Pollutants, including fecal matter, can be transported to waterways through runoff during or after rain events. Land use partially determines how quickly pollutants are transported. For example, grasses or vegetated land tend to soak up rainfall, increasing infiltration into the ground and reducing runoff into waterways. Alternatively, impervious surfaces like streets, rooftops, sidewalks, parking lots, or driveways, do not allow for water infiltration, therefore, increase the amount of runoff. Lands that support animals like cattle, hogs, or horses, can also be a source of bacteria. This is especially true if the animals enter waterways for drinking or if heavy rains wash manure from the land into receiving water.

Another source of bacterial pollution in waterways originates from point sources. Point sources are defined by the U.S EPA as "any single identifiable source of pollution from which pollutants are discharged," such as broken pipes. Additionally, bacteria can enter water from illicit connections, stormwater outfalls, and sewage discharge from wastewater treatment plants. These discharges may occur due to large rain events, power failures, or maintenance problems due to the excessive volume of water entering the plant.

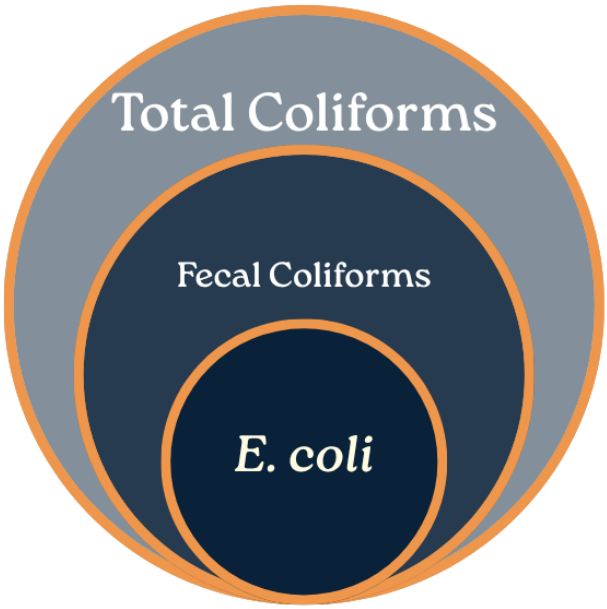
How do Bacteria Pose a Risk to Human Health?

High levels of bacteria may pose a risk to human health. Higher bacteria levels lead to greater potential for human health risks like gastroenteritis, a condition indicated by vomiting, diarrhea, fever, nausea, and stomachache; skin disease; and respiratory eye, ear, nose, throat, and skin infections. *E. coli* alone is not generally a cause for alarm; excessive levels of *E. coli* may indicate the presence of harmful bacteria and pathogens like:

- E. coli 0157
- *Salmonella* and *shigella* (causes of gastrointestinal illnesses)
- *Psuedomonas aeruginosa* (causes swimmer's ear or dermatitis)
- Protozoans such as *Cryptosporidium* and *Giardia*
- Viruses like hepatitis A

Relationship Between Fecal Coliforms and *E. coli*

Fecal coliforms are bacteria that originate from fecal matter. *E. coli* is a species of bacteria within the fecal coliform group that originate from the waste of warm-blooded animals and makes up about 60 – 80% of the fecal coliform group. Refer to the graphic below for a general organization of coliform bacteria.



The U.S. EPA recommended conversion factor between fecal coliform and *E. coli* is 126/200, which results in an *E. coli*/fecal coliform (EC/FC) ratio of 0.63. However, a recent US Geological Survey paper showed the EC/FC ratio to be closer to 0.77.

Because *E. coli* is more closely related to gastrointestinal illness caused by swimming than fecal coliforms, the U.S. EPA has recommended *E. coli* as an appropriate indicator species for assessing potential health risks for recreational waters.

Georgia Bacterial Standards

The state of Georgia uses fecal coliform as the water quality standards for bacterial contamination. Refer to the graphic below for an outline of fecal coliform standards set by the state. These standards were established to support the requirement by the U.S. EPA to protect all waters for use of primary contact, recreation, or swimming. Acceptable fecal coliform levels are determined by "use classification" for all bodies of water.

Georgia Fecal Coliform Standards

Use Classification	Bacteria Levels (Fecal Coliform)	
	30- Day Geometric Mean (cfu/100 mL)	Maximum (cfu/100 mL)
Drinking Water Requiring Treatment	1,000 (Nov-April) 200 (May-Oct)	4,000 (Nov-April)
Recreation	200 (Freshwater) 100 (Coastal)	--
Fishing Coastal Fishing	1,000 (Nov-April) 200 (May-Oct)	4,000 (Nov-April)
Scenic River	No alteration of natural water quality	
Wild River	No alteration of natural water quality	

Recommended *E. coli* Standards

The U.S. EPA recommended *E. coli* as the freshwater quality criterion for bacteria in the *Ambient Water Quality Criteria for Bacteria* document published in 1986. This was a departure from earlier recommendations of total coliform and fecal coliform. The *E. coli* recommendation resulted from epidemiological studies that found *E. coli* to be statistically correlated with swimming-related gastrointestinal illnesses.

In recreational waters like those for swimming and water skiing (full body contact), the EPA recommends an *E. coli* limit less than or equal to 126 cfu/100 mL (colony forming units per 100 milliliters of water). These numbers are based on a geometric mean or a one-time measurement less than or equal to 235 cfu/100 mL. The U.S. EPA recommends a set of standards for *E. coli* in freshwater bodies as a single maximum allowable count. These rates correspond to an acceptable risk level of 8 people out of 1,00 becoming ill. See table below for U.S. EPA *E. coli* level recommendations.

There will always be fecal bacteria in the environment, regardless of watershed management practices. However, if high levels of *E. coli* are regularly found on a long-term basis, you should alert water agencies, health departments, local governments, watershed groups, and community leaders to identify and correct the problem.

Recommended *E. coli* Standards for Recreational Waters

	Designated Swimming	Moderate Swimming Area	Light Swimming Area	Infrequent Swimming Area
E. coli (cfu/100 mL)	<235	<298	<410	<576

High Bacterial Count Results

Weather and seasonal effects influence bacteria levels. This natural variability makes bacterial concentrations in waterways difficult to predict. Bacteria numbers often increase following a heavy storm or excessive runoff event. *E. coli* is often more prevalent in turbid waters because they live in soil and can attach to sediment particles. Bacteria can remain in stream bed sediment for long periods of time. If the stream bed has been stirred up from rainfall or increased flow, samples may have elevated bacteria levels. Avoid disturbing the sediment as you wade into the stream for this reason and always

collect samples upstream. When collecting from several sites on one stream, always collect the furthest downstream sample first and proceed upstream.

Several environmental factors can affect bacteria survival in waterbodies. *E. coli* levels are often higher in summer months and lower in winter months. Similarly, high levels of *E. coli* may be found in warmer water because it is adapted to living in the intestines of warm-blooded animals and persists in water closer to its optimal growth temperature. However, ultraviolet light from the sun can kill bacteria in clear waters.

Additional Water Quality

A comprehensive assessment of water quality should include monitoring for other water quality indicators. A combination of biological, chemical, and physical factors may indicate if a waterbody is impaired. How the water is used (drinking, swimming, etc.) may influence which characteristics are used to determine water quality.

In addition to bacteria, Adopt-A-Stream recommends chemical testing for basic parameters like dissolved oxygen, temperature, conductivity, and pH which are discussed in the *Chemical Monitoring* manual. Other common water quality measurements include clarity and nutrients (particularly nitrogen and phosphorus) and macroinvertebrate biological communities. Recording visual observations of the monitoring site provides vital information for assessing a waterbody. Adopt-A-Stream offers a *Visual Stream Survey* manual and accompanying workshop to provide volunteers with a background in visual waterway assessment.

Most land use activities that impact waterways can be pinpointed by analyzing human activity within the watershed. Determining land use provides a more complete picture of pollution sources impacting the waterbody. The Watershed Survey and Map Assessment located in the *Getting to Know Your Watershed* manual provides guidance on activities and land uses to look for.

Georgia Adopt-A-Stream has developed several manuals to assist volunteers in watershed monitoring efforts. Information on testing for chemical, macroinvertebrate, and visual stream parameters are available in the manuals *Introduction to Water Quality Monitoring, Getting to Know Your Watershed, Visual Stream Survey, Macroinvertebrate Monitoring, Chemical Monitoring, Amphibian Monitoring, and Wetland Monitoring* on the Georgia Adopt-A-Stream website.

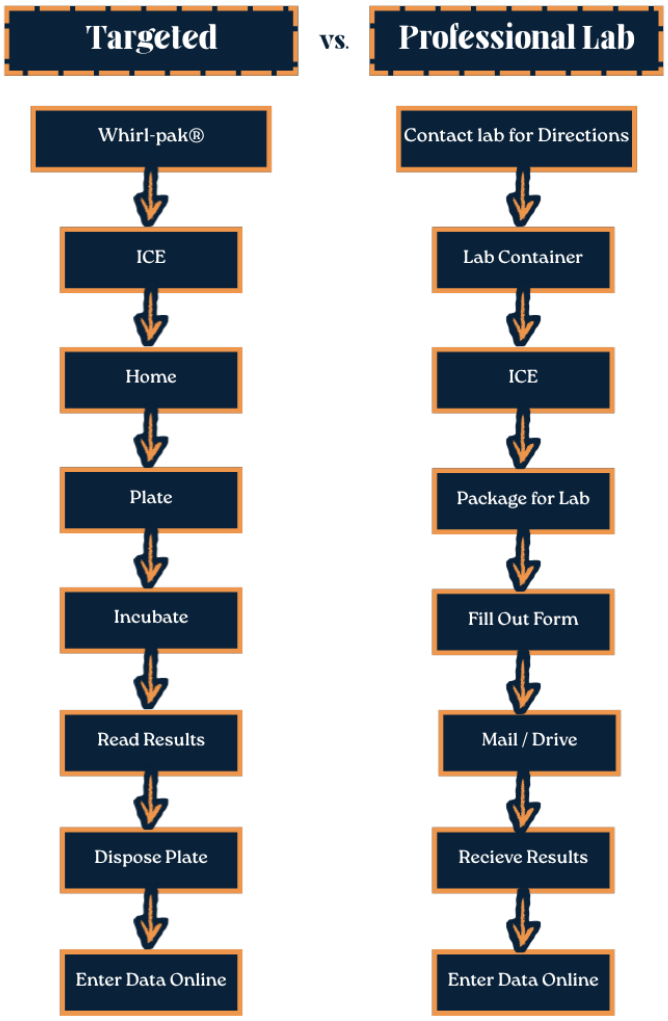
SAMPLING FOR BACTERIA

When to Sample

Georgia Adopt-A-Stream recommends sampling for *E. coli* at least once a month during dry weather conditions. Time of day should also remain consistent. These factors help with comparing data over time. The number of times required to sample varies and depends on the participants' goals. To get well rounded information and ensure the ease of interpreting data, sample monthly.

Choosing a Sampling Method

Volunteers may opt for processing samples from home using 3M™ Petrifilm™ plates and an incubator, or by packing and shipping samples to a professional laboratory. Many studies show that using the 3M™ Petrifilm™ method is as effective as professional lab methods. It is also a more practical and cost-effective method for volunteers. Refer to the Georgia Adopt-A-Stream website for information about professional quality lab data and our partnership with the UGA Cooperative Extension Service's water quality lab. Refer to the flow chart for the general process of water samples taken from the field.



Equipment Needed for Bacterial Monitoring

After a thorough review of 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 must be maintained at a specific temperature. For updated equipment specifications and ordering information, visit the Monitoring Resources page on our website.

Field Sample Equipment Checklist

- ☐ *Bacterial Data Form*
- ☐ Rubber boots, waders, or old tennis shoes
- ☐ Bucket with a rope or grab sample pole (if sampling from a bridge)
- ☐ Whirl-Pak® bags (pre-labeled with site and collection information)
- ☐ Latex or vinyl gloves
- ☐ Permanent marker
- ☐ Disinfected cooler with ice
- ☐ First aid kit
- ☐ *Who to Call List* (found on the AAS website)
- ☐ Trash bag for litter

Plating and Incubating Equipment Checklist

- ☐ *Bacterial Data Form*
- ☐ Cup to hold Whirl-Pak® bags
- ☐ 3M™ Petrifilm™ *E. coli* plates
- ☐ 1 mL fixed-volume pipettor & sterile tips
- ☐ Clean space for sample process with good lighting
- ☐ Incubator
- ☐ Digital thermometer
- ☐ Permanent marker
- ☐ Latex or vinyl gloves
- ☐ Safety glasses
- ☐ 10% Bleach solution, OR Lysol spray/disinfectant
- ☐ Watertight bag for sample disposal

Directions for Bacteria Monitoring Using 3M™ Petrifilm™

Preparing the Blank Sample

For each sampling event (a day of sampling up to 10 sites), fill a Whirl-Pak® bag with distilled water at the first sample site to serve as the blank/control. Having a blank when sampling is necessary to serve as a control. A control ensures that you are practicing sterile techniques and preventing contamination. The blank sample bag should remain with you during your sampling event and go through the same environmental exposures as you stream samples. If sampling at multiple sites, prepare one blank for every cooler, up to 10 sites. The blank is later plated and analyzed alongside the stream samples in the lab. Lab analysis of the blank should result in a zero reading for bacteria. If the blank is contaminated, all samples must be discarded; no data can be submitted for these samples. Prior to preparing the control sample, label the Whirl-Pak® bag with the word "BLANK," date and time collected, and the sample collector.

Follow the steps below for preparing a blank sample:

1. Label one Whirl-Pak® bag with a permanent marker for the blank/control
    a. Include the date, time, and name of the person sampling
2. Put on latex gloves and remove perforated seal from the top of the Whirl-Pak® bag
    a. **IMPORTANT!** Do not touch the inside of the Whirl-Pak® bag 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 about 2/3 of the way full with distilled water
5. Grab the ends of the yellow twist ties and "whirl" or spin the bag away from you until it is tight. Cross and twist the ties to close the bag
6. Ensure the bag is securely closed by inverting the bag to test its seal (no water should leak out)
7. Immediately place the Whirl-Pak® bag into a properly disinfected cooler with ice and store there throughout the sampling event alongside the other samples collected

Collecting Samples in the Field

Follow the steps below when collecting samples in the field:

1. Label a new Whirl-Pak® bag with the sample and site information using a permanent marker
2. Put on latex or vinyl gloves and remove the perforated seal from the top of the Whirl-Pak® bag
    a. **IMPORTANT!** Do not touch the inside of the Whirl-Pak® bag 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. Allow water to fill the bag until it is about 2/3 of the way full
    a. Squeeze out excess water if the bag is too full
    b. Always collect samples upstream from where you are standing
    c. If sampling from a bridge, use a rope tied to a small, disinfected bucket to get the sample
5. Grab the ends of the yellow twist ties and "whirl" or spin the bag away from you until tight. Cross and twist the ties to close the bag.
6. Ensure the bag is securely sealed by inverting it to test the seal (no water should leak out)
7. Immediately place the Whirl-Pak® bag into the cooler with the blank
8. Holding time for samples on ice or refrigerated is no more than 24 hours
9. Properly dispose of gloves and perforated seal tabs

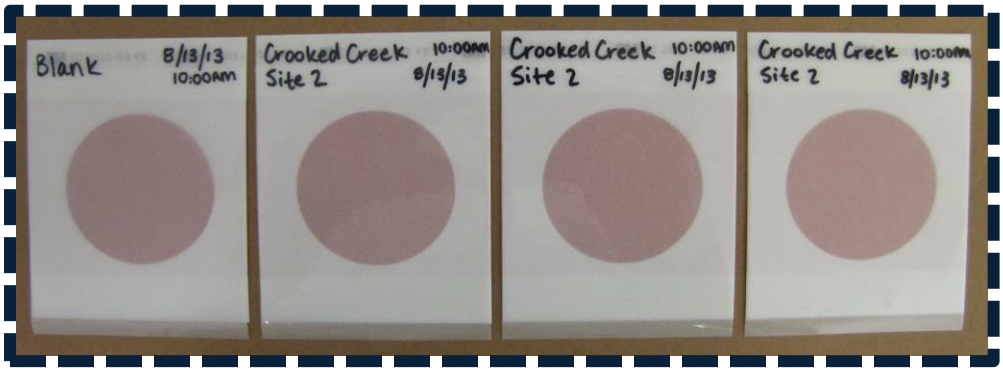
Plating Samples

Note: Turn incubator on before plating to ensure it will reach the correct incubation temperature. To allow them to thaw, remove Petrifilm™ plates from the freezer before plating for 10-15 minutes. Process the blank/control sample first (use one plate) and then follow with stream samples (use three plates). There will be four plates used in total, and one pipette tip for each sample bag.

Follow the steps below when plating samples:

1. Disinfect working area and let dry before beginning
2. Put on latex gloves
    a. **Gloves should always be worn when handling plates** (even when going to read them)
3. Label all four plates correctly (one blank and three site samples), and lay them on a clean, flat surface.
    a. Plate labels should include stream name, site number, incubation start time, and the date

b. See figure below for correctly labelled plates



4. Gently shake or invert Whirl-Pak® bag to ensure an evenly mixed sample
5. Place the Whirl-Pak® bag in a cup to keep from spilling and use the white tabs to open the bag.
    a. **IMPORTANT!** Do not touch the inside of the Whirl-Pak® bag as this will contaminate your sample and could alter the results
6. Carefully remove a pipette tip from sterile container
    a. Do not touch the pipette tip inside of the sterile container
    b. Practice caution to ensure the tip does not become contaminated
7. Pipette 1 mL of the sample using a fixed-volume pipettor
8. Lift the top film of the Petrifilm™ plate and dispense 1 mL of sample on the center of the circular plate
9. Slowly roll the top film down onto the sample until the plate is completely covered to prevent trapping air bubbles
    a. Do not touch the center of the Petrifilm™ plate with the pipette tip or fingers
10. If necessary, distribute the sample evenly by using a 3M™ spreader or slightly tilting the Petrifilm™ plate back and forth
    a. Tilting too much will cause the sample to pour out of the plate
11. Leave plate undisturbed for at least one minute to allow the gel to solidify before placing it in the incubator
12. Repeat: Plate two more samples from the same bag for a total of three plates per sample site.

Incubating a Sample

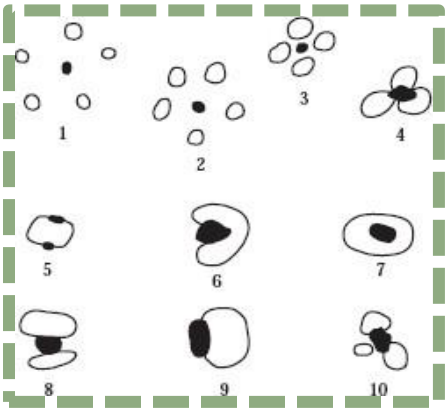
1. Turn incubator on prior to plating to ensure it is at the correct temperature before plates go in. Place the incubator lid on top.
2. Insert a thermometer into the incubator
3. When incubator reaches a temperature of 35°C ± 1°C, place the processed Petrifilm™ plates (including the blanks) into the 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.

5. **Incubate for 24 ± 1 hour at 35°C ± 1°C**
6. After 24 hours, remove plates (with gloves on) and count *E. coli* colonies
7. Record the minimum and maximum temperatures that are displayed on the thermometer after incubating, as well as the in and out times of the plates
8. Record all data on the *Bacterial Data Form*
9. Dispose of plates by spraying them with disinfectant, placing them in a sealed zip lock back, and discarding them in the trash

Reading the Results

When reading Petrifilm™ plates, ***E. coli*** colonies appear blue to red-blue and are closely associated with entrapped gas. **General coliform** colonies appear bright red and are 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 are growing more than halfway off the medium** of the plate (outside the circle). Refer to the image on the right for gas bubble patterns associated with gas producing colonies.



Only count 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 colony forming units per 100 milliliters of water sample (cfu/100 mL). Each Petrifilm™ plate holes 1 mL 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 or more *E. coli* colonies, many gas bubbles, and, depending on the gel, a color from red to purple-blue. High concentrations of *E. coli* will cause the entire growth area to turn into a deep purple-blue color. The plate will be filled with so many colonies that there is barely any empty space 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.

Following the steps below to determine the number of colony forming units (cfu) per 100 mL of water sample:

1. Count the number of *E. coli* colonies on all three plates and add them together

    a. **Example:** Plate 1 has 6 colonies, plate 2 has 7 colonies, and plate 3 has 8 colonies

    b. **Example: $6 + 7 + 8 = 21$ *colonies***

2. Take the total number of colonies, divided by the number of plates used to find the average number of colonies

    a. $\frac{Plate\ 1+Plate\ 2+Plate\ 3}{3\ Plates} = Average\ Number\ of\ Colonies$

    b. **Example:** $\frac{21\ Colonies}{3\ Plates} = 7\ colonies$

3. Multiple the average number of colonies by 100 to determine the number of colony forming units per 100 mL of sample

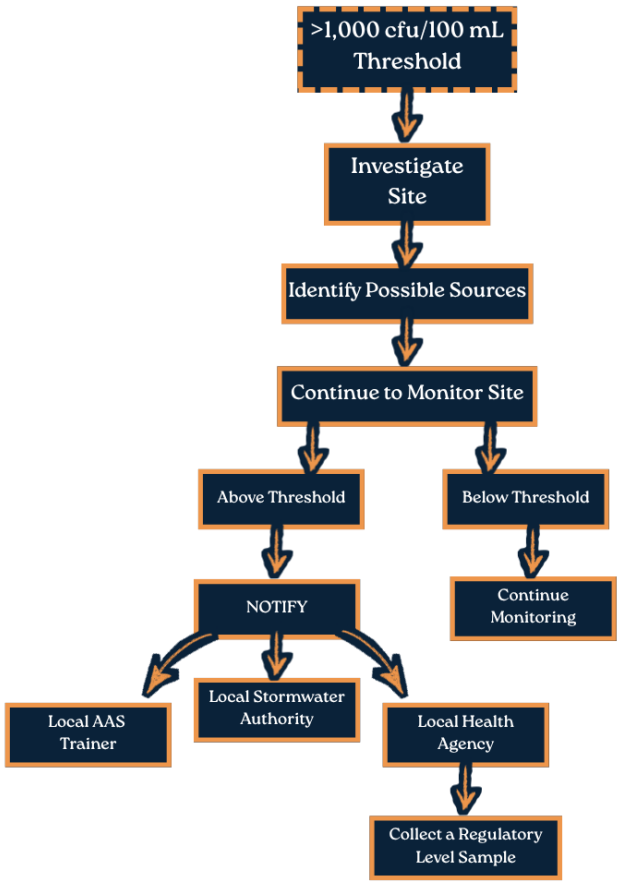
    a. $\left(\frac{Plate\ 1+Plate\ 2+Plate\ 3}{3\ Plates}\right) 100 = \frac{cfu}{100mL}$

    b. **Example:** $\frac{7cfu}{1mL} \times 100\ mL = 700\frac{cfu}{100mL}$

Getting High Levels of Bacteria

Georgia Adopt-A-Stream recommends sampling for *E. coli* at least once a month during normal flow conditions. It is common to find high bacteria levels in urban areas. *E. coli* counts (cfu/100 mL) that exceed 235 cfu/100 mL are considered "high" and should be closely monitored. When counts exceed the 1,000 cfu/100 mL threshold, they warrant special action. A count of 235 cfu/100 mL correlates to approximately 8 people out of 1,000 getting sick, whereas a count of 1,000 cfu/100 mL correlates to about 14 people out of 1,000 becoming sick.

If "high" bacteria counts are found, it may be a one-time occurrence. This information is useful, but before taking further action, return to the site and take more samples. Pay attention to anything out of the ordinary at the sight. Note the presence of animals and any unusual odors. Walk through the banks and look for any obvious sources of pollution and note the current weather conditions. Continue sampling and contact local health agencies if numbers remain high. Be sure to wear gloves while sampling and to thoroughly wash hands afterwards.



If bacteria levels are regularly about the 1,000 cfu/100 mL threshold, contact your local Adopt-A-Stream trainer to find the cause. Alert local watershed groups or agencies about the monitoring efforts and results. They may be able to help find the possible source of *E. coli* pollution. Refer to the graphic if consistent high *E. coli* counts are detected.

Using guidance provided by the U.S. 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 information on state fecal coliform standards and U.S. EPA *E. coli* standards can be found on the Georgia Environmental Protection Division and EPA websites, respectively.

Source Tracking

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

When used correctly, BST methodologies can turn nonpoint (diffuse) source pollution into point source pollution. Current BST research is being driven by the recent implementation of the Total Maximum Daily Load (TMDL) concept by the U.S. EPA. BST methods represent the best tools for determining fecal pollution in water and should be an integral part of any project involving 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.

Molecular (genotype) and biochemical (phenotype) BST methods are under development. The most well-known example of BTS methods is DNA finger printing, but numerous methods show potential. Most researchers believe that a combination of BST methods will be needed to provide the most accurate and reliable source identification answers. It is doubtful that any one BST methods will emerge as the “best” method for all situations.

While this is not a procedure volunteers will conduct, it is one to be aware of, and a possible step that state agencies may take. As of now, it is still an emerging and costly technology, but it is being incorporated more in government agencies.

Equipment Usage

Equipment Maintenance

When dealing with bacteria, it is crucial to practice sterile techniques to avoid contamination of samples. Consider the following when maintaining sampling equipment:

- ❖ Store pipette tips in a sterile container
- ❖ Use a new, sterile pipette tip when plating from different water samples
- ❖ Disinfect the sample-holding cooler with a 10% bleach solution before and after each use
- ❖ Wash hands thoroughly with soap and water before and after plating
- ❖ Use latex or vinyl gloves when handling equipment

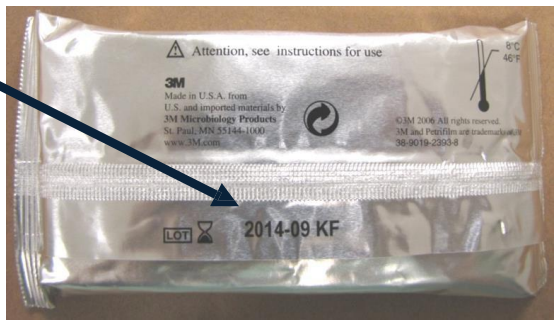
Using a Fixed-Volume Pipettor with Sterile Tips

When plating a sample, use fixed-volume pipettors and ensure tips are secured in a sterile container. **Note:** Always agitate the water sample before drawing a sample with a pipette.

Follow the steps below to use a fixed-volume pipettor:

1. Add a tip to pipettor
2. Depress the "plunger to the first stage
3. Dip the tip in the water sample
4. Release the "plunger" slowly to reach the ready position
    a. The water sample will be filled in the tip
    b. Be sure there are no air bubbles
5. Depress the "plunger" gently to the first stage
    a. Solution will be dispensed but a small amount of liquid will remain in the tip
6. After a pause, depress the "plunger" to the second stage
    a. The small amount of liquid left in the tip will now be dispensed
7. Release the "plunger" to reach the ready position

3M™ Petrifilm™ Storage



Store un-opened Petrifilm™ plate pouches in the refrigerator. Once the pouch is opened, avoid any moisture encountering the plates. Store opened Petrifilm™ plate pouches in a re-sealable plastic bag and place them in the freezer. Place a weight on the top of the bag to ensure plates are lying flat. Exposure of Petrifilm™ plates to temperatures greater than 25°C (77°F), and/or humidity greater than or equal to 50% relative humidity can affect the performance of the plates. Opened pouches that have been stored properly will last until their expiration date. Refer to the image below to locate the expiration date on the back of the pouch. Discard any plates with discoloration.

Disposal Techniques

After counting the bacterial colonies, plates must be disinfected before disposal. Any of the following disinfectants may be used:

- A 10% bleach solution
- Lysol spray
- Rubbing alcohol or ethanol

Wear gloves and add one of the above disinfectants to each plate. If working with bleach, do not allow the solution to come into contact with clothes or skin. Use a re-sealable plastic bag to hold plates. Allow the contents to mix for five minutes before disposing of it in the trash.

Other Sampling Methods

For more information comparing different processing methods, click the link to view the *Volunteer monitoring of E. coli in streams of the upper Midwestern United States: a comparison of methods.*

UGA Cooperative Extension Service

Adopt-A-Stream partners with UGA Cooperative Extension Service to process *E. coli* samples. The UGA lab requires specific, pre-approved containers provided by their lab. Contact UGA Cooperative Extension Service for general recommendations on mailing samples. Volunteers have the option of using any state-certified lab to process samples.

IDEXX Colisure

Due to the costs associated with using IDEXX Colisure, volunteers typically do not opt to use it. However, its accuracy when compared with laboratory analyses was as good as both methods. **Follow the steps below to use the IDEXX Colisure:**

Preparation and Setup

1. Turn on IDEXX Quanti-Tray® Sealer
2. Label Quanti-Trays® using a permanent marker
    a. Include site ID, date and time of sample collection, and the sample number

Preparing the Sample

1. Water samples are collected in 100 mL IDEXX bottles by filling the bottle up to the 100 mL graduation
2. Add Colisure reagent and two drops of anti-foam solution to the sample
3. Mix thoroughly until reagent is dissolved
4. Pour sample into Quanti-Tray®

5. Place Quanti-Tray ®on rubber insert and seal with Quanti-Tray® Sealer
6. Remove from back of sealer as soon as sealing is complete

Incubation and Interpretation

1. Incubate at 35°C for 24-48 hours
2. After incubation period, read results
    a. Wells containing total coliforms will turn from yellow to magenta
    b. Wells containing *E. coli* will turn from yellow to magenta and fluoresce under UV radiation
    c. If wells appear pink or orange, return tray to incubator and reexamine it in four hours
3. After positive wells are counted, refer to the Most Probable Number (MPN) table to determine the total coliform MPN and *E. coli* MPN

Sample Disposal

1. Quanti-Trays® must be sterilized by autoclaving
2. Store used trays in large resealable plastic bags
3. Return for disposal during each subsequent sample transfer

IDEXX Colilert

Due to the costs associated with using IDEXX Colilert, volunteers typically do not opt to use it. However, its accuracy when compared with laboratory analyses was as good as both methods. **Follow the steps below to use the IDEXX Colilert:**

Preparation and Setup

1. Turn on IDEXX Quanti-Tray® Sealer
2. Label Quanti-Trays® using a permanent marker
    a. Label should include the site ID, date and time of sample collection, and sample number

Preparing the Sample

1. Water samples are collected in 100 mL plastic IDEXX bottles by filling the bottles to the 100 mL graduation
2. Add Colilert reagent and two drops of anti-foam solution into the sample
3. Mix thoroughly until reagent is dissolved
4. Pour sample into Quanti-Tray®
5. Place Quanti-Tray® on rubber insert and seal with the Quanti-Tray® Sealer
6. Remove from back of sealer as soon as sealing is complete

Incubation and Interpretation

1. Incubate at 35°C for 24-48 hours

2. After incubation period, read results
    a. Wells containing total coliforms will turn from clear to yellow
    b. Wells containing *E. coli* will turn from clear to yellow and fluoresce under UV radiation
3. After positive wells are counted, refer to the Most Probable Number (MPN) table to determine the total coliform MPN and *E. coli* MPN

Sample Disposal

    c. Quanti-Trays® must be sterilized by autoclaving
    d. Store used trays in large resealable plastic bags
    e. Return for disposal during each subsequent sample transfer

Packing & Shipping Water Samples

All samples taken must be analyzed within 24 hours. If shipping water samples to an analytical lab, collect them early in the week and no later than Wednesday to allow time for the lab to process them prior to the weekend. Arrange with mail carrier prior to sampling to ensure samples will be collected promptly and delivered within 24 hours. On the day of sampling, sample early in the day to ensure they are shipping out the afternoon of the same day. To secure bottles for shipping, ensure there are no leaks and are kept cold. Consider the following:

- Line the shipping container with a plastic garbage bag to prevent leaks
- Prevent cross-contamination from leaks or breakage by sealing each sample in its own plastic bag
- Pack samples with ice or ice packs
- Use a sealed plastic cooler or specialized water sample shipping container
- Fill out documentation completely
    - Sampling form, chain of custody form, and other necessary paperwork
- Seal paperwork in a zipped storage bag and place in inside of container before sealing
- Attach the pre-addressed, pre-paid mailing label and ship overnight

*Please note that UGA Cooperative Extension Service requires specific, pre-approved containers provided by the UGA lab. Contact UGA Cooperative Extension Service for general recommendations on mailing samples.

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