Bacterial Monitoring

Learning Module #12

WATER DAWGS

Agenda

Time	Length	Activity
1:00	**20 min**	**Opening Activity**
1:20	**1 hour**	***E. coli* in the Chattahoochee**
2:20	*15 min*	*BREAK*
2:35	**20 min**	**All about bacteria!**
2:55	**1 hour**	**Bacterial Monitoring Protocols**
3:55	*15 min*	*BREAK*
4:10	**20 min**	**Closing Activity**

Opening Activity

Instructions:

1. Write your response to the questions on the lesson worksheet.

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Opening Activity

- **Question 1: What do you already know about *E. coli*?**

For example, why is it a problem? How do humans contract *E. coli*? Have you ever heard about it in the news? Feel free to write down information that you learned this morning, or things you already knew.

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E. coli in the Chattahoochee River

- In the next activity, we are going to be exploring *E. coli* concentrations in the Chattahoochee River.

- Do you know where the Chattahoochee River is located?

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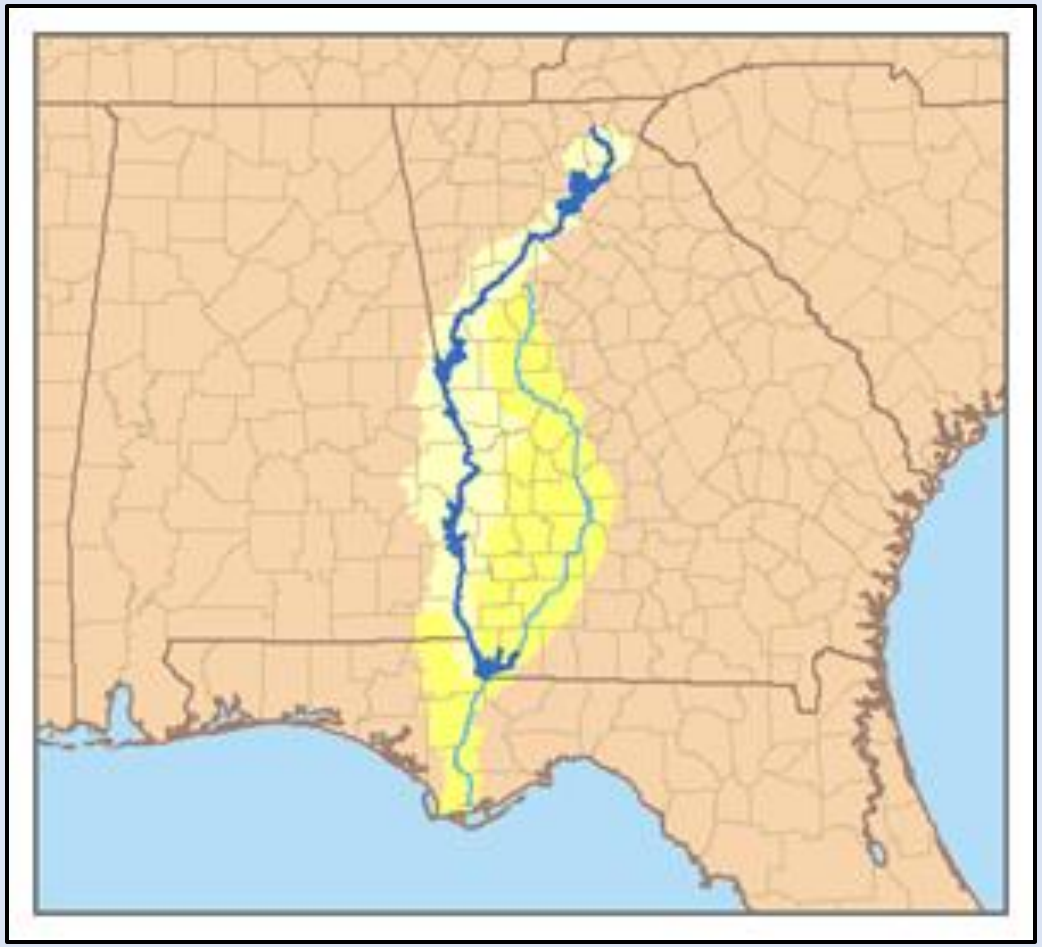
E. coli in the Chattahoochee River



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E. coli in the Chattahoochee River

News video from 7 October 2021 (WSB Atlanta):

https://www.wsbtv.com/news/local/atlanta/rain-raises-e-coli-levels-chattahoochee-dangerously-high-levels/3PYOXDOHYJFBHKSYS4QK7VXE6I/

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E. coli in the Chattahoochee River - Discussion questions

1. How high were the *E. coli* concentrations in the Chattahoochee in October 2021?

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E. coli in the Chattahoochee River - Discussion questions

1. How high were the *E. coli* concentrations in the Chattahoochee in October 2021?
2. How is *E. coli* and other bacteria entering the Chattahoochee? (hint: there are two ways)

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E. coli in the Chattahoochee River - Discussion questions

1. How high were the *E. coli* concentrations in the Chattahoochee in October 2021?
2. How is *E. coli* and other bacteria entering the Chattahoochee? (hint: there are two ways)
3. Why do swimmers need to avoid the Chattahoochee when *E. coli* levels are above the designated standard?

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E. coli in the Chattahoochee River - Activity

- Next, we are going to visit the Chattahoochee RiverKeeper's BacteriALERT page so we can explore real-time *E. coli* data!

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E. coli in the Chattahoochee River - Activity

Directions to access BacteriALERT website:

- Go to Google
- Type in “Chattahoochee RiverKeeper”
- Click on website (Chattahoochee.org)
- Along the top panel on the main page, scroll over “Our Work”
- Under “Our Work”, click “Water Quality Monitoring”
- Scroll to the bottom of the page. Click on “View BacteriALERT Data”

Once we access the website, you will have 40 min to follow the guiding directions/questions on your lesson worksheet.

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Take a break!

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All about bacteria!

- Next, we are going to learn more about bacteria and *E. coli*.
- There are no guided notes today, but feel free to take your own notes and/or ask questions during the presentation!

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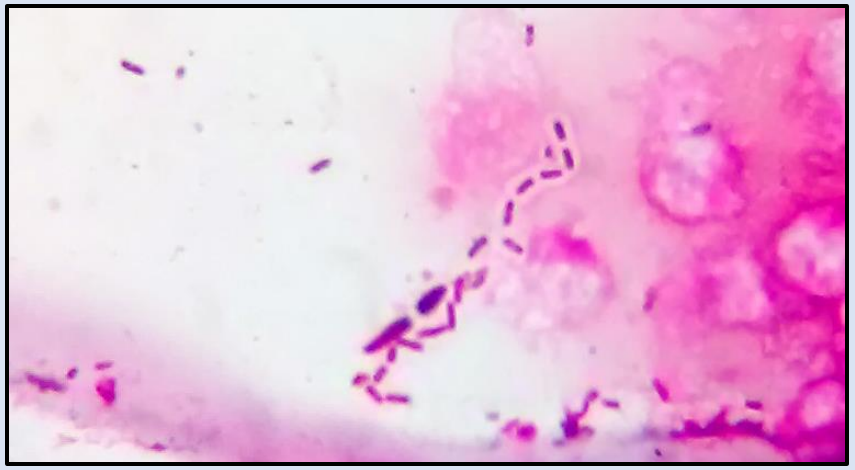
All about Bacteria!

What are bacteria?

- Microscopic-single celled organisms
- They can survive and adapt to almost all conditions present on earth
- Most bacteria are beneficial and responsible for important environmental processes like decomposition, nutrient cycling, and the breakdown of environmental toxins
- However, some bacteria are pathogenic (or disease-causing)



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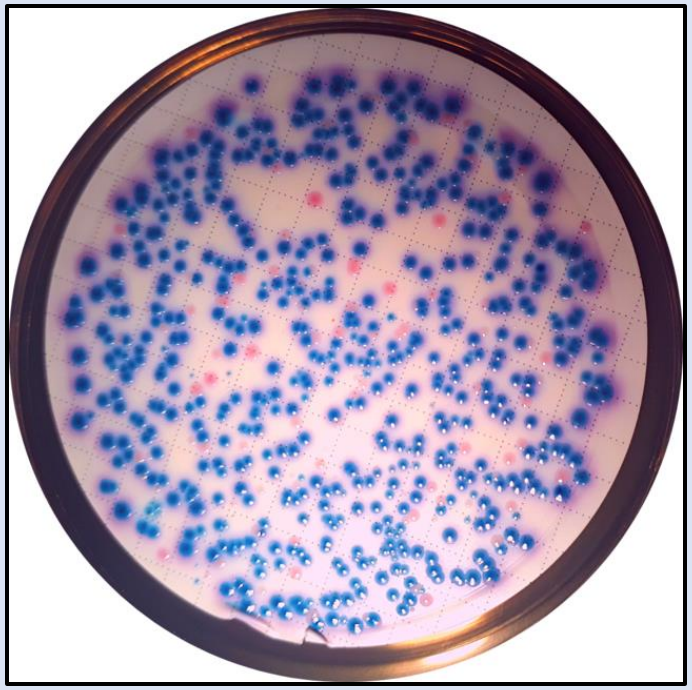
All about Bacteria!



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What are coliform bacteria?

- Coliform bacteria are members of the Enterobacteriaceae family
- Some are found naturally in soil, some types live in the intestinal tract of warm-blooded animals
- The types that are found in human and animal wastes are called fecal coliform bacteria

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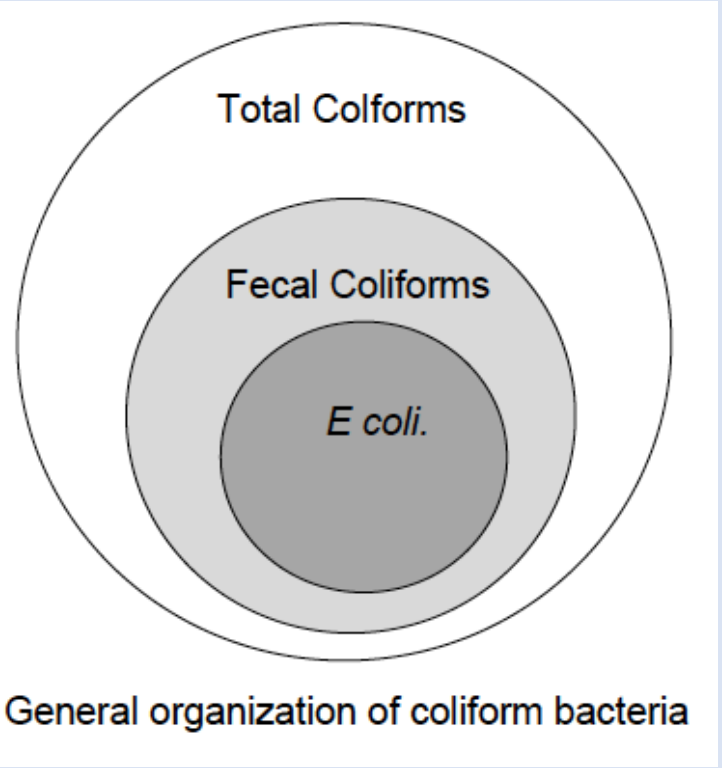
All about Bacteria!

What is *E. coli*?

- *Escherichia coli* (*E. coli*) is one subgroup of fecal coliform bacteria
- Even within this species, there are many strains. Some are harmless and some are pathogenic
- *E. coli* is a useful indicator bacteria. It is an indicator of fecal contamination – but their presence does not necessarily mean pathogens are present! (That is because many strains of *E. coli* are non-pathogenic!!)
- However, if *E. coli* bacteria are present at high concentrations, there may be risk to human health



General organization of coliform bacteria

Photo credit: from Georgia Adopt-A-Stream Bacterial Monitoring Manual

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All about Bacteria!

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How does *E. coli* get into waterways?

- Non-point source pollution
  - Animal fecal matter is on land (dog parks, dairy farms, land application of animal waste, poultry operations, geese in parks, etc.). When it rains, runoff can carry this fecal matter into streams and rivers.
- Point source pollution
  - Failing septic tanks
  - Leaking sewer lines
  - Wastewater treatment plants

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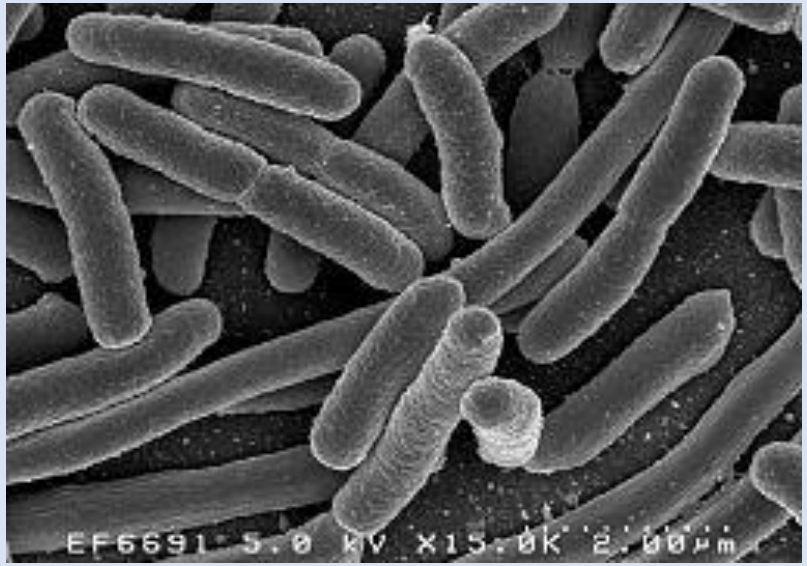
All about Bacteria!

How does *E. coli* affect human health?

- Higher the bacterial levels = higher risk of gastroenteritis
  - Vomiting, diarrhea, fever, nausea, stomachache; skin infections; and respiratory, eye, ears, nose, throat infections
- Excessive levels of *E. coli* may indicate presence of harmful pathogens such as:
  - *E. coli* 0157
  - Salmonella
  - Shigella
  - *Cryptosporidium*
  - Giardia
  - Hepatitis A



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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

(from US EPA 1986, 2002a)

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Bacterial Monitoring Protocols

There are 5 steps to the bacterial monitoring process:

1. Prepping the blank/control sample
2. Collecting site samples in the field
3. Plating your samples
4. Incubating
5. Reading the results

Steps 1 and 2 have already been done for you. We will be completing Steps 3 and 4 today with our study stream!

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Bacterial Monitoring Protocols

Materials needed

<u>To collect:</u>

- Bacterial Data Form
- Boots
- Whirl-pak bags
- Gloves
- Sharpie
- Cooler with Ice

<u>To plate/incubate:</u>

- Cup to hold Whirl-pak bags
- 3M Petrifilm E. coli plates
- 1mL pipette and sterile tips
- Incubator
- Sharpie
- Safety glasses
- Lysol/bleach spray

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Step 1: Prepping the Blank/Control sample

What is a blank/control sample?

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For bacterial monitoring, it’s when the scientist fills a sample bag with distilled water instead of sample water.

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Why might we need a blank/control sample?

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Step 1: Prepping the Blank/Control sample

What is a blank/control sample?

For bacterial monitoring, it's when the scientist fills a sample bag with distilled water instead of sample water.

Why might we need a blank/control sample?

A control sample will ensure that you are practicing sterile (clean) techniques and that your samples are not contaminated.

Lab analysis of the blank should result in zero reading for bacteria. If it is contaminated, you will need to discard all of the samples!

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Step 1: Prepping the Blank/Control sample

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.

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Step 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.

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Step 2: Collecting site samples in the field

Why do you want to sample upstream from where you are standing?

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Step 2: Collecting site samples in the field

Why do you want to sample upstream from where you are standing?

To make sure you are not collecting water after sediment has been disturbed.

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Pipette Review

The procedure for Step 3 involves using a pipette.

Has anyone used a pipette before?

What is a pipette and why do we use them?

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Pipette Review

How to use a pipette:

https://www.youtube.com/watch?v=8Afh_0IAfrQ

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Pipette Review

Let’s practice using a pipette with distilled water!

Steps:

1. Set/check the volume
2. Put a tip on your pipette
3. Depress the plunger (1st stop)
4. Withdraw the solution
5. Expulsion of the solution (1st stop then 2nd stop)
6. Discard the tip

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Step 3: Plating your samples

What is petrifilm?

- Petrifilm is a plate that is covered with a certain agar. The agar turns the E. coli colonies blue and the coliform colonies red. The top film captures gas produced by the colonies.

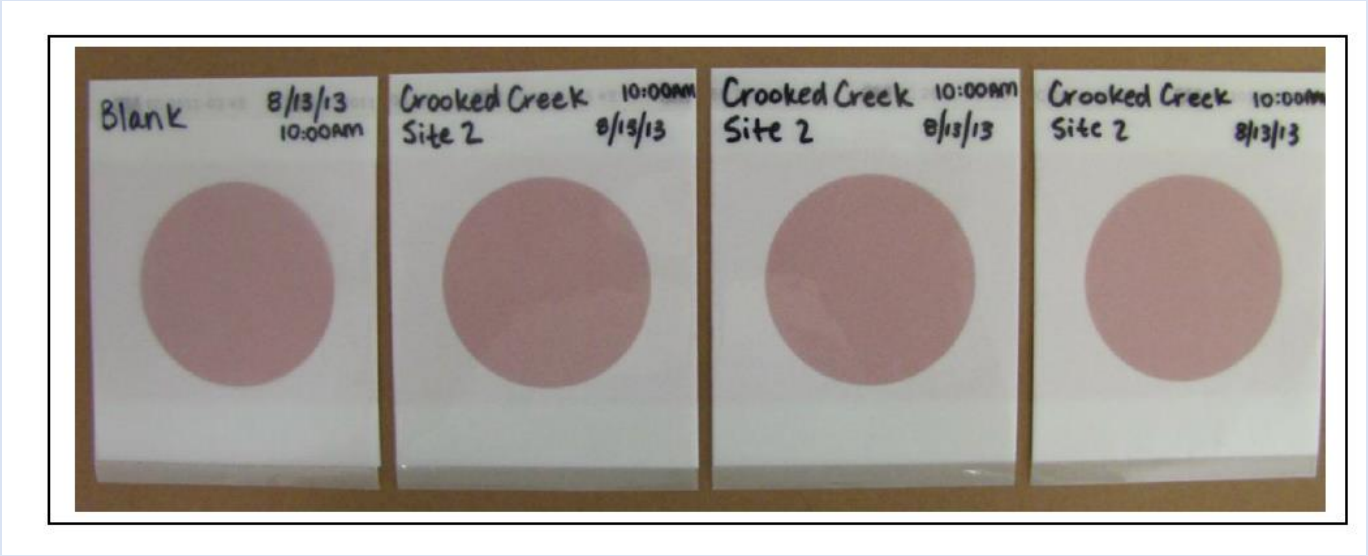

Photo credit: Georgia Adopt-A-Stream directions for Bacterial Monitoring

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Step 3: Plating your samples

We will split into three groups. Each group will plate one sample from our study stream (Lily Branch).

On your group's plate, make sure you have labeled:

- Stream name
- Rep #
- Incubation start date
- Incubation start time

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Step 3: Plating your samples

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.

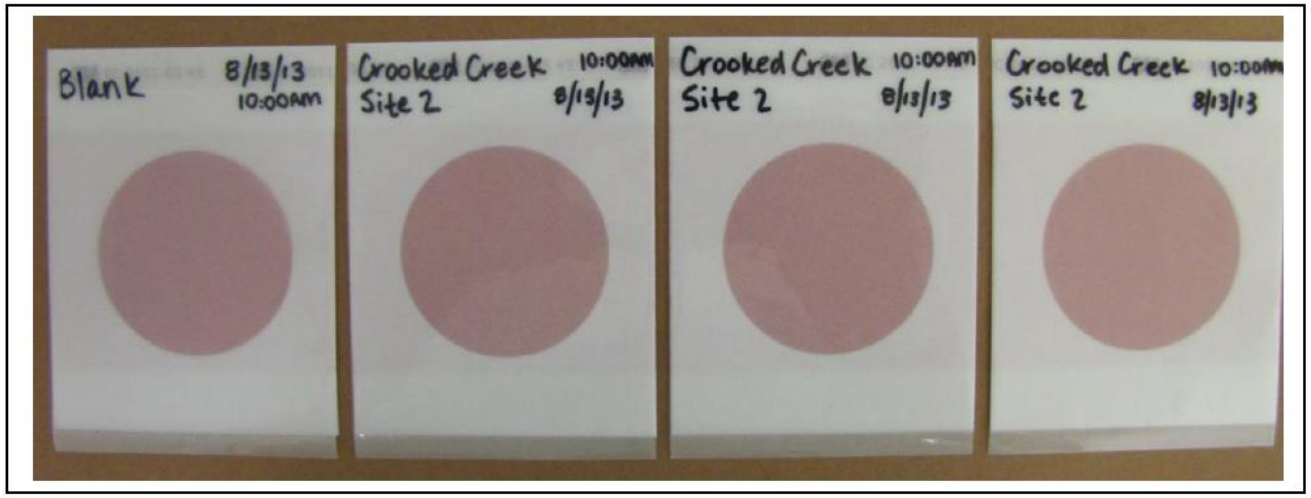

Photo credit: Georgia Adopt-A-Stream directions for Bacterial Monitoring

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Step 3: Plating your samples

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.

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Step 4: Incubating your samples

What is an incubator? Why do we use them?

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Step 4: Incubating your samples

What is an incubator? Why do we use them?

An incubator is a heated, insulated box used to grow and maintain microbiological or cell cultures. The incubator maintains optimal temperature, humidity and gaseous content of the atmosphere inside.

Photo credit: By Uncle Zooey - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=48998391

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Step 4: Incubating your samples

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.

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Don’t forget to clean up when you’re done!
Use 10% bleach solution!

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Take a break!

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Closing Activity

Instructions:

1. Write your response to the questions on your lesson worksheet.

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Closing Activity

Scenario: Imagine that you are volunteering for the Upper Oconee Watershed Network (a citizen science group). You are measuring *E. coli* levels in rivers and streams around Athens during early fall months. You go to sample a popular swimming hole, which is at the intersection of Barber and McNutt creeks. The swimming hole is located near a park where many people walk their dogs. You find *E. coli* concentrations in the swimming hole to be 450 cfu/100 mL. You look back and past UOWN data and realize this site has a history of having high (above EPA standard) *E. coli* concentrations.

<u>Answer the following questions:</u>

1. How would you plan to communicate your findings with local citizens?
2. You are asked to give advice to the park managers about what they could do to help decrease *E. coli* concentrations to the creeks and swimming hole. What would you suggest?

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