RIVANNA
CONSERVATION ALLIANCE

StreamWatch Citizen Science Program

Bacteria Monitor Manual

<u>If any issues arise, never hesitate to call Anne @ 434-249-5306</u>

1. Introduction

What Are Bacteria?
Bacteria are single-celled organisms that occur in a variety of forms and have a wide range of properties. Some cause disease while others decompose decaying organic material and serve as food for other organisms in the food chain.

Why Monitor Bacteria?
Pathogenic (disease-causing) bacteria, viruses, and protozoans are often found in fecal waste. These pathogens can cause a variety of illnesses and diseases when ingested during recreational contact or consumed in contaminated water and shellfish. Fecal waste from humans or other warm-blooded animals may enter a waterbody from various sources including faulty wastewater treatment plants, livestock, malfunctioning septic systems, untreated sewage discharge, pets, stormwater runoff, wildlife, or boat waste. Since it is not practical to monitor for every pathogen, "indicator" species are monitored. The presence of indicator species suggests the presence of fecal waste that may include pathogenic microorganisms that pose a health risk. In addition to the possible health risk associated with elevated levels of fecal material, it can also cause cloudy water, nutrient enrichment, unpleasant odors, and an increased oxygen demand.

Which Bacterial Indicator Do We Use?
Bacterial indicators commonly measured by professional and volunteer monitoring programs include fecal coliform, *Escherichia coli* (*E. coli*) and *enterococci*. These indicators are normally prevalent in the fecal waste of warm-blooded animals and humans. For the purposes of volunteer monitoring, DEQ recommends testing for *E. coli* or enterococci bacteria because they are monitored by the agency for recreational waters.

E. coli
E. coli is a species within the fecal coliform group that is specifically associated with the fecal waste of warm-blooded animals. In freshwater, E. coli corresponds more closely with swimming-related illnesses than fecal coliform.

Coliscan Easygel
Coliscan Easygel (Micrology Labs) is simple to use and relatively inexpensive. The Coliscan Easygel method measures total coliforms and E. coli. A water sample is added to a liquid medium and poured onto a treated Petri dish.
The Coliscan Easygel method was compared to laboratory analysis and found to be an acceptable tool for screening purposes although the data cannot be used directly by DEQ for water quality assessments. This method is important because it can assist you in locating "hot spots" for fecal contamination and target areas for more extensive monitoring.

What Do Your Bacteria Results Mean?
Water quality standards for *E.coli* and *Enterococci* were adopted by Virginia and became effective in January 2003. Water is deemed unsafe for recreation when *E. coli* (freshwater) is above 235 colonies /100 ml water.

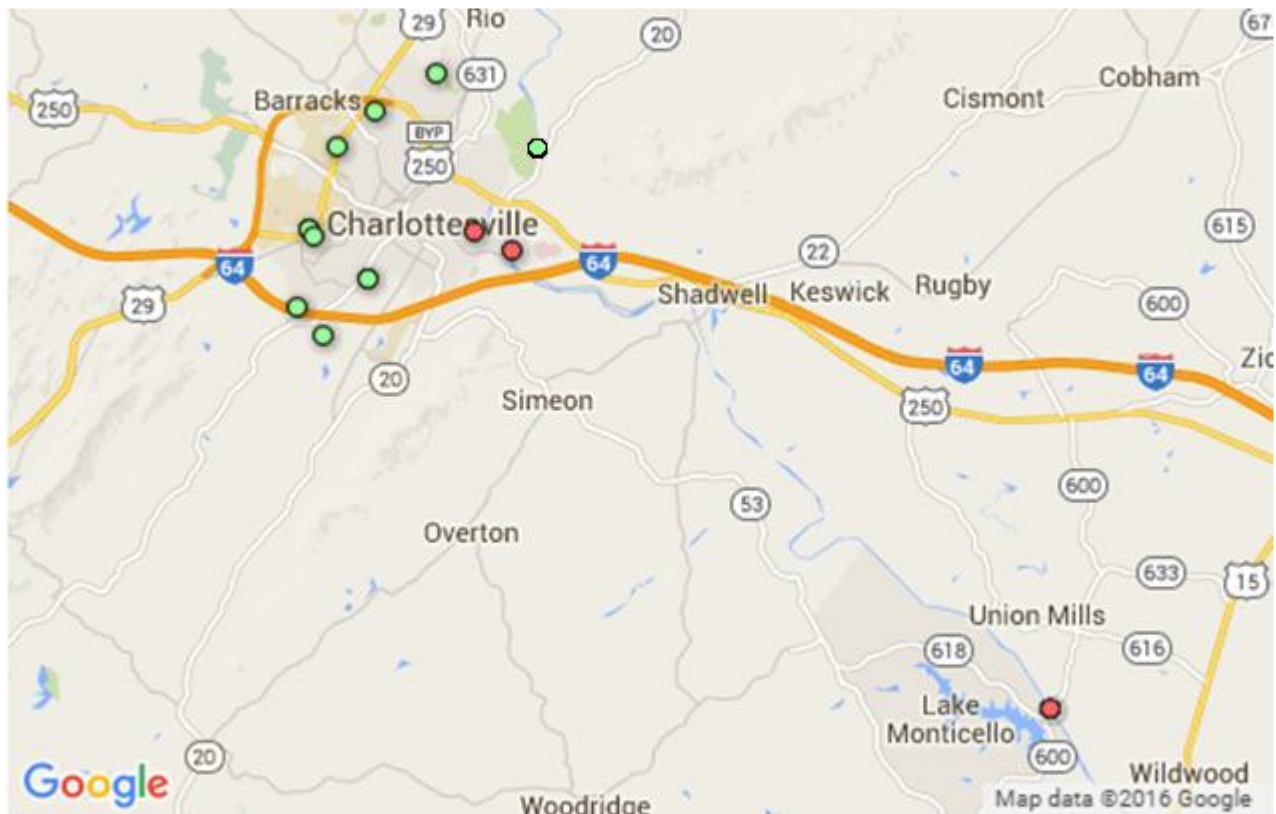
2. Safety

When sampling, use alcohol sanitizer to keep hands clean as the water may be contaminated. If sampling along a streamside or in the stream, **do not sample when water levels are dangerously high**, such as after a major rainstorm. Do not wade into water that is swift flowing or unusually high. It is critical to obtain a representative sample. The water sample should be obtained from the main flow of the water body. In small streams (assuming it is safe), it is best to wade into the main flow of the stream. **If the flow is too high to wade into** use a sampling bucket with rope and toss it into the flow to collect your water sample.

3. Sampling Time Frame

Volunteers will collect samples during daylight hours until 2pm on the first Saturday of each month. Collection before 2 pm will allow time for the sample to incubate for 24hrs so that it can be analyzed the next day at reasonable hour. It is up to teammates to coordinate schedules and designate when and who will sample on Saturday, and when (24 hrs later) and who will read the sample on Sunday.

4. Sample Locations



Site Code	Site Name	Latitude	Longitude
BSC01	Biscuit Run West of Southern Pkwy	38.0037	-78.5087
MDC01	Meade Creek @ Meade Park	38.02763	-78.4650877
MSV05	Moore's Creek Upper at Azalea Park	38.01028	-78.51667
MWC13	Meadow Creek @ Brandywine Dr.	38.063705	-78.475853
MWC11	Meadow Creek @ Meadowbrook Gardens	38.054943	-78.494134
MWC12	Meadow Creek @ Copeley Rd.	38.046887	-78.504979
RVN09	Rivanna River @ Riverview Park	38.02335	-78.45388
RVN10	Rivanna River @ Crofton	37.9182	-78.2975
RVN11	Rivanna River @ Darden Towe Park	38.045668	-78.451295
RCK02	Rock Creek @ 5th St SW	38.016846	-78.495795
LDC01	Lodge Creek @ 5th St SW	38.01674	-78.495829
LDC03	Lodge Creek near JPA	38.026649	-78.51153
XLC01	Lodge Creek East Tributary	38.028302	-78.513082
XLD01	Lodge Creek West Tributary	38.028144	-78.513082

5. Monitor Kit Checklist

Before you go out into the field. Make sure you have everything in your kit that you need:

- Empty (and sterile) sample bottles
- Turbidity sample bottle
- Thermometer
- Data sheet
- Pencils & Sharpie
- Frozen icepack

6. Take A Sample

It is critical to obtain a representative sample. The water sample should be obtained from the main flow of the water body. In small streams (assuming it is safe), it is best to wade into the main flow of the stream. Collecting the sample from the side of the stream using a sampling bucket is best for larger or deeper streams, provided the streambank is safe to sample from.

Part 1- Bacteria Sample:

1. Walk upstream a short distance with minimal disturbance of the sediment.
2. Facing upstream, open the bacteria sample bottle (**small white bottle**). Be sure to not contaminate the cap.
3. Carefully fill a sterile white bottle by submerging it below the surface as you move the bottle away from your body in an upstream direction. This method ensures any bacteria from hands or boots (or boat) does not enter the bottle. Avoid letting mud and other debris enter the sample bottle. Fill the bottle about to ¾ full.
4. Cap the bacteria sample bottle, dry it as needed with towel, and label it with the station ID using a waterproof pen. For split samples, use -1 or -2 at the end of the station ID value to note which split sample it is.
5. Immediately place the sample bottle on ice for transport to the lab.

Part 2- Turbidity Sample:

1. Use the bottle labeled "turbidity sample bottle" in your kit. This bottle gets reused each month.
2. Uncap the bottle and with the same U motion, submerge the bottle below the water and move it away from you in an upstream direction.
3. Empty the bottle downstream of you.
4. Repeat steps **2.** and **3.** two more times. We need to thoroughly rinse the bottle with the sample.
5. Repeat step **2.** collecting your sample and cap the bottle.
6. Put it on ice in your pack.

Part 3- Temperature:

1. Have someone not in the stream take an air temperature reading. Ideally out of direct sunlight. Wait for the temperature reading to stabilize before recording it on the data sheet.
2. While remaining in the stream take a water temperature measurement where you took your two samples. Place the metal tip in the water and wait for the temperature reading to stabilize. Record the temperature in Celsius.

Part 4 – Fill in Data Sheet

Be sure to completely fill in your data sheet. Note any strange odors, color, or debris in the stream. Also, please mark down your volunteer hours including your travel time.

Bucket Method

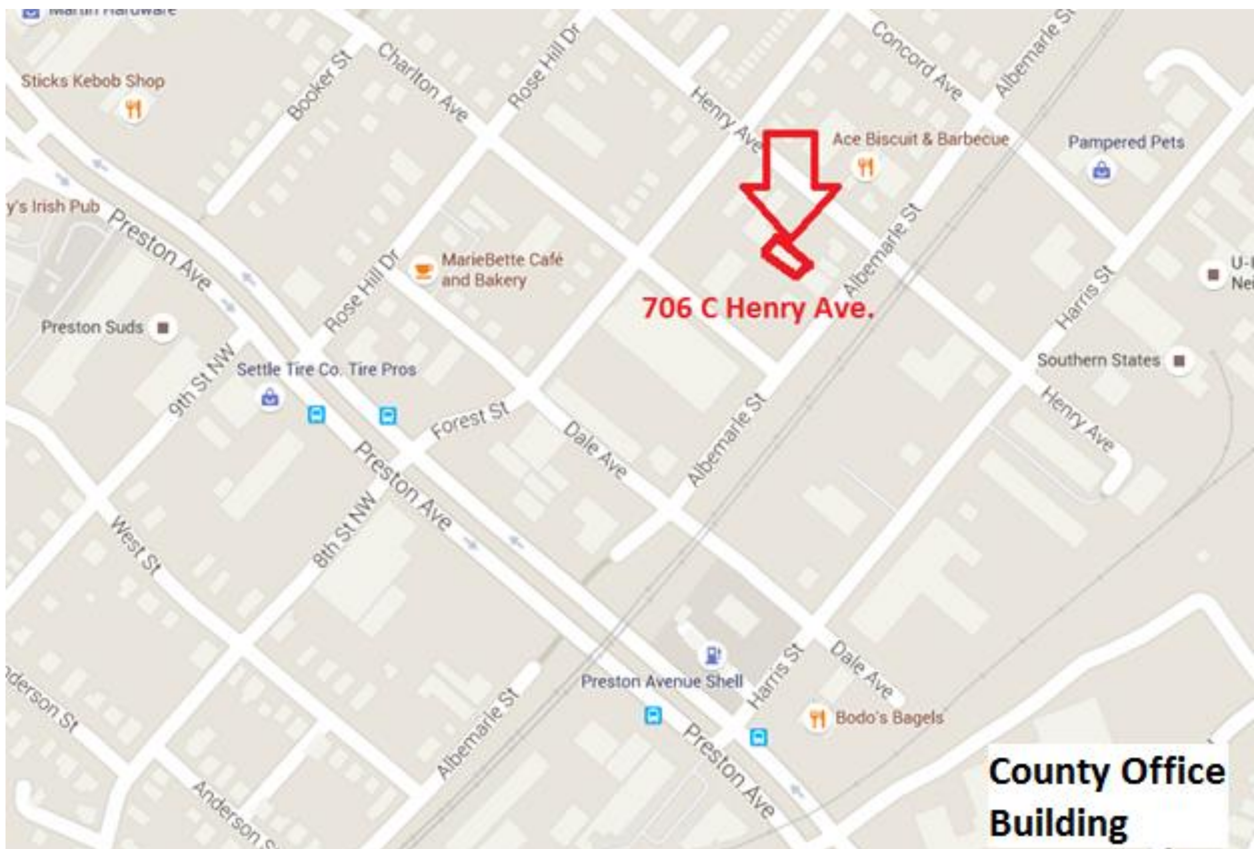
If after a storm the stream is unsafe to wade into, you may use a bucket to collect your water samples.

1. Toss a clean (not sterile) bucket from the side of the stream using rope and partially fill the bucket.
2. Retrieve the bucket and swirl the sample water and dump the contents away from the sample site.

3. Toss the bucket once more and fill part way with a water sample. Try not to collect excessive sediment, mud, or other debris in the bucket.
4. Retrieve the bucket.
5. Using a U motion, fill the sample bottle by moving it through the water from one side of the bucket to the other in such a way to prevent water from near the hand from entering the bottle. Fill the bottle about ¾ full.
6. Cap the bottle, dry it as needed with towel, and label it with the station ID using a waterproof pen. For split samples, use -1 or -2 at the end of the station ID value to note which split sample it is.

7. Lab Procedures

After you have collected your sample, take it to the field lab located at 706 C Henry St. across from Ace BBQ. There is a code box on the door that has a key inside. To get the code to the box please email Anne at AnneDunckel@rivannariver.org or call her at 434-249-5306. Please be sure to lock the door and replace the key when you leave.



Step 1. Plate your bacteria sample

1. Plug in the incubator.
2. Label the bottom (smaller, taller piece) of the Petri dish provided in the Coliscan Easygel kit using a permanent marker. It is best to label the dishes using small lettering on the outer rim of the dish. The minimum information needed should be the site ID number, date, and replicate number (if needed).

3. Retrieve the sample bottles from your field kit. Samples should not be frozen, but kept cool on ice or ice packs and must be tested as soon as collected.
4. **Mix the water sample in the sample bottle by shaking or swirling for several seconds.**
5. Transfer the 3 milliliters to a bottle of Coliscan medium using a sterile pipette. Do not reuse the pipette for other sample bottles. Only work with one sample at a time to avoid confusing bottles.
6. **Gently swirl** the bottle of Coliscan media for several seconds so that it mixes with the sample water. **<u>Do not shake</u> the bottle** as this will cause the medium to foam and makes reading the colonies difficult during the counting phase.
7. Open the labeled Petri dish and pour the entire contents of the bottle into bottom portion of the Petri dish. It is important to perform this step on a level surface so the solution forms an even layer across the plate. DO NOT leave the Petri dish interior open to the air for longer than necessary as mold and other contaminates can enter and contaminate the plate.
8. If needed, gently swirl the Petri dish so the solution covers the entire plate. Allow the solution to solidify (approximately 60 - 90 minutes) prior to incubation. While the plate is setting you can do your turbidity measurement.
9. Incubate the Petri dishes upside down for 24 hours at 35° - 40° Celsius. This is approximately 95° - 105° F.
10. Note the time the sample went into the incubator on your data sheet. Also, you can put a sticky tag on the plate with the time you put it in. Be sure to coordinate schedules with your team so that someone can be available to read the sample within 24 hours (+/- 1 hour) of it going inside the incubator.
11. Place your bacteria sample bottle labeled with the sample ID # and date in the refrigerator.

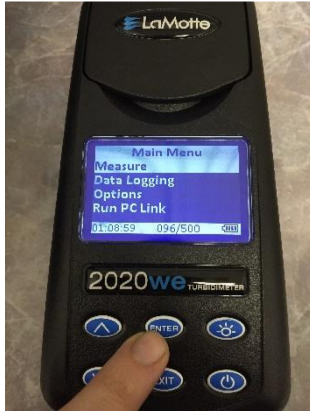
Step 2. Turbidity Measurement

The turbidity meter lives in this case.

A. Calibration (to be performed each time that the turbidity meter is used)

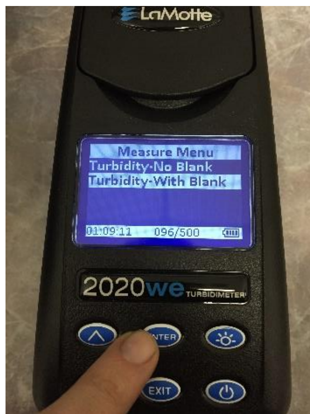
1. Pull out the meter and place it flat on the table. Unless the battery is dead there is no need to have it plugged in.

2. Turn on turbidity meter.

3. 

Follow menu prompts and select "Measure". Text is dark and the line is highlighted when it is selected.

4. press ENTER

5. 

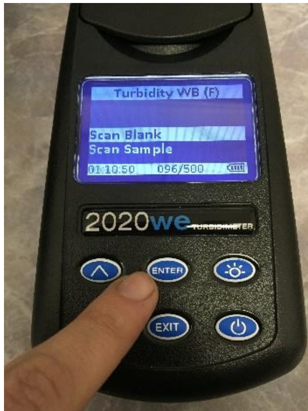
Select "Turbidity –With Blank",

6. Press ENTER.

7. Turbidity is measured by light penetration through the sample, and results can be skewed by smudges or stray fingerprints left on a vial. Before inserting any vials into the turbidity meter, wipe clean with Kimwipe to remove any moisture or fingerprints.

8. Insert vial labeled “blank” into the meter, with white vertical line on vial positioned to face user.

9. Close turbidity meter cap.

10. 

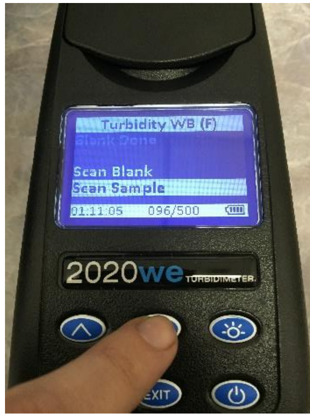
Select “Scan Blank.”

11. Press ENTER.

12. After a few seconds, once unit has completed “blank” calibration, remove “blank” vial.

13. Insert the vial containing “10 NTU” standard, again with white vertical line on the vial positioned to face user and after again wiping with Kimwipe.

14. Close turbidity meter cap.

15. 

Select "Scan Sample"

16. Press ENTER.

17. If the reported turbidity value equals the standard value of 10 NTU, calibration is complete and you may now continue to the steps below titled **Turbidity Measurement**.

18. If the reported value **does not equal** the standard value of 10 NTU follow these steps.

19.

Press the down arrow key to highlight "Calibrate"

20. Press ENTER.

21.

Use the arrow keys to adjust the value to 10.00 NTU,

22. Press ENTER.

23.

"Set Calibration" will be highlighted and you must

24. Press ENTER one more time to complete calibration.

B. Turbidity Measurement

1. Gently shake your turbidity sample to mix contents thoroughly.

2. Use an empty vial in the turbidity meter case and rinse it with your sample using the **Rule of Three.** That means you will fill the bottle with your sample and dump it into the liquid waste bucket 3 times before filling it with the final sample that you will measure.

3. After the 3rd rinse, fill the vial to the white horizontal line.

4. Make sure you are on the "Turbidity – WB" page. If not go back to the main menu, select "Measure", then "Turbidity – With Blank".

5. Before inserting any vials into the turbidity meter, wipe clean with Kimwipes to remove any moisture, oils or fingerprints.

6.
Insert vial labeled "blank" into the meter, with the white vertical line on vial positioned to face user.

7. Close turbidity meter cap.

8.

Select "Scan Blank"

9. Press ENTER.

10. Once unit has completed "blank" calibration, remove the "blank" vial.

11. Insert the vial containing river water, again with white vertical line on vial positioned to face user.

12. Close turbidity meter cap

13.

Select "Scan Sample"

14. Press ENTER.

15.

Record reported turbidity value on data log sheet.

16. Take out your sample and dump it in the liquid waste bucket.

17. Turn the machine off and put everything back in its place. Put your empty turbidity sample bottle back in your sample kit for use again next month.

Step 3. Data Analysis

1. The bacteria plate should be analyzed 24 hours (+/- 1 hour) after the plate was put in the incubator.

2. Use white or graph paper as a background to make identifications easier. If there are a large number of colonies (>100), drawing quadrants on the plate using a sharpie can help to count colonies.

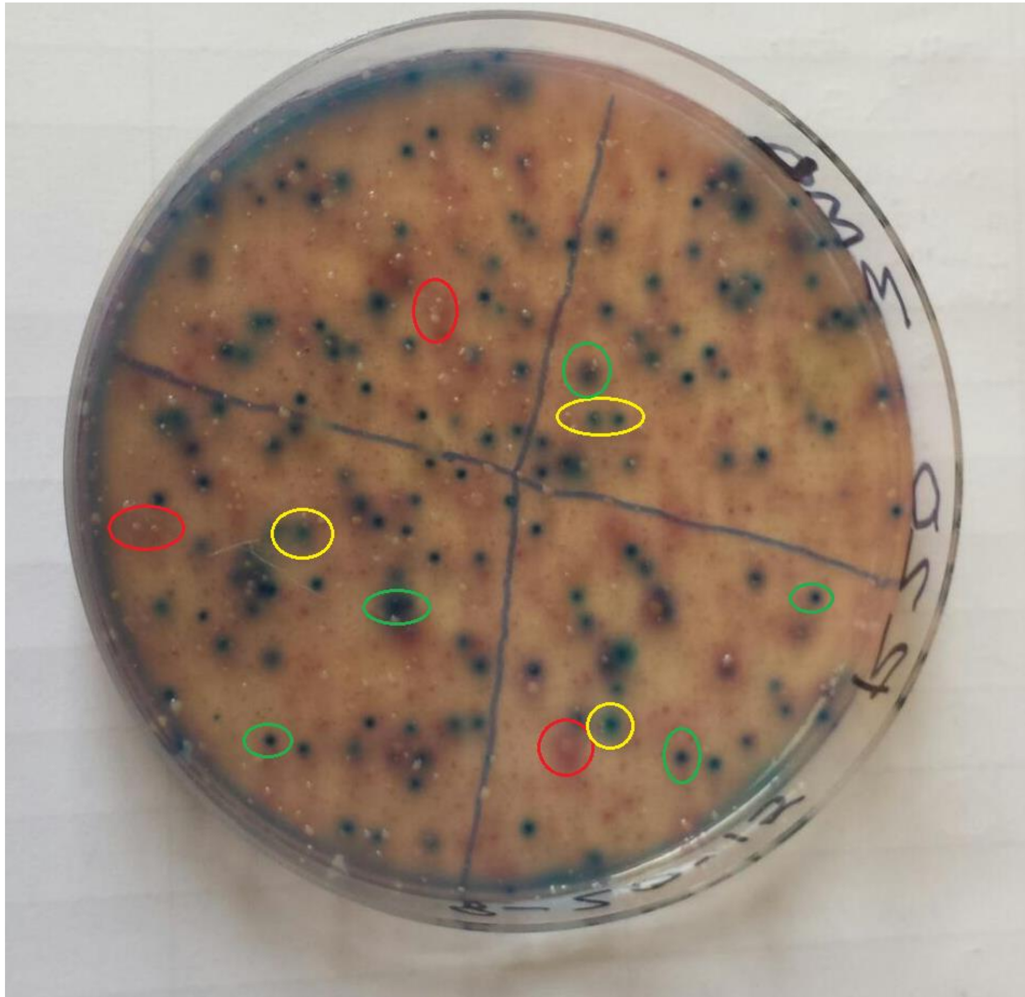
3. Count the number of **dark blue to royal purple** colonies on each plate and record this number in field sheet. Do not count teal colored or pink – dark red colonies or colonies colored anything other than **dark blue to royal purple**. If you are unsure of some colonies, you can use the microscope with the light on to confirm the color.

4. If more than 60 E.coli colonies are counted, draw a quadrants on the back of the plate and count each quadrant, write the number for each quadrant down, and then recount it again. If you have another person with you, have both of you count independently and then compare numbers. Recount multiple times until your numbers agree. If the plate is too numerous to count, ~500 or more, don't worry about getting an accurate number and write **too numerous to count** on the data sheet.

5. Calculate the number of E.coli cells (CFU) per 100 milliliters and record on the data form.

$$\textbf{\# E. Coli colonies counted} \div \textbf{3mL} \times \textbf{100} = \underline{\qquad}\textbf{CFU/100mL}$$

6. Take a picture of the plate with the camera along with a piece of paper that has the sample ID, date, and time written on it.



Examples: Red circles show white and pink colonies that are **not counted.** Yellow circles show teal colonies that could be confused for royal blue colonies. Green circles show dark blue to royal purple colonies that **are counted.**

Step 4. Disposal of Waste & Clean-up

1. Dispose used pipettes and sample bottles in blue recycle bucket.
2. Wipe down the area where plates were prepared or counted with a Lysol® wipe. Throw the wipe in the trash can.
3. After recording the results on the field sheets, open and place dishes in a 1 gallon plastic Ziploc™ style bag and pour two to four ounces rubbing alcohol into the bag and seal the bag shut. Shake bag for 30 seconds and dispose as normal trash.
4. If your sample is the last one out, unplug the incubator.
5. Place ice pack in the freezer for use next month.

Step 5. Data recording & reporting

1. Make sure all aspects of your field data sheet are filled out including your turbidity and bacteria counts.
2. If you have not taken a photo of the plate, do so using the provided camera. Take the picture with a piece of paper that has the sample ID, date, and time written on it also in the frame.
3. Place your completed field datasheet in the clear binder pocket. A volunteer will collect them on Monday and input the data.

Thank you!

The data you collect is extremely helpful for many people including people who swim in the river, and officials at the City of Charlottesville, UVa, Albemarle County, and Fluvanna County.

Data will be posted the Monday following the weekend sample at:

www.rivannariver.org/bacteria