RIVANNA
CONSERVATION ALLIANCE

Bacteria Monitor Manual

***Updated February, 2020**

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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.

Colilert Testing
Colilert method (Micrology Labs) was added to RCA's testing protocols in 2017. This method measures Most Probable Number (MPN) of bacteria in a sample. Medium is added to the 100 mL sample of water, and the sample is sealed into a Quanti-Tray and incubated before reading.
RCA has been certified by DEQ to use the Colilert Method, and the resulting data can be used directly by DEQ for water quality assessments.

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 CFU (colony forming units) /100 ml water. With the Colilert technique, this standard is 235 MPN (most probable number).

2. Safety

- When possible, sample in teams of at least two people in case a sampler is injured or needs assistance.
- Check the local weather forecast before going out to confirm safe sampling conditions.
- Always notify someone when heading out to sample including the sample location and expected return time and provide a cell phone number if available.
- When sampling, use gloves or have alcohol sanitizer available to keep hands clean as the water may be contaminated due to sewage.
- Wear an orange or similarly colored brightly colored reflective vest or similar garments when sampling from bridges or when sampling in the woods during hunting season.
- If sampling along a streamside or in the stream, **do not sample when water levels are dangerously high**, such as during or after a major rainstorm. Do not wade into water that is swift flowing or over knee high.
- Do not sample if lightning is in the area or in the middle of a thunderstorm.
- If the flow is too high to wade into, use a sampling pole to collect your water sample.

Remember, it is not worth sacrificing personal safety for a sample. If a sampler feels uncomfortable with a site, look for another location to sample from.

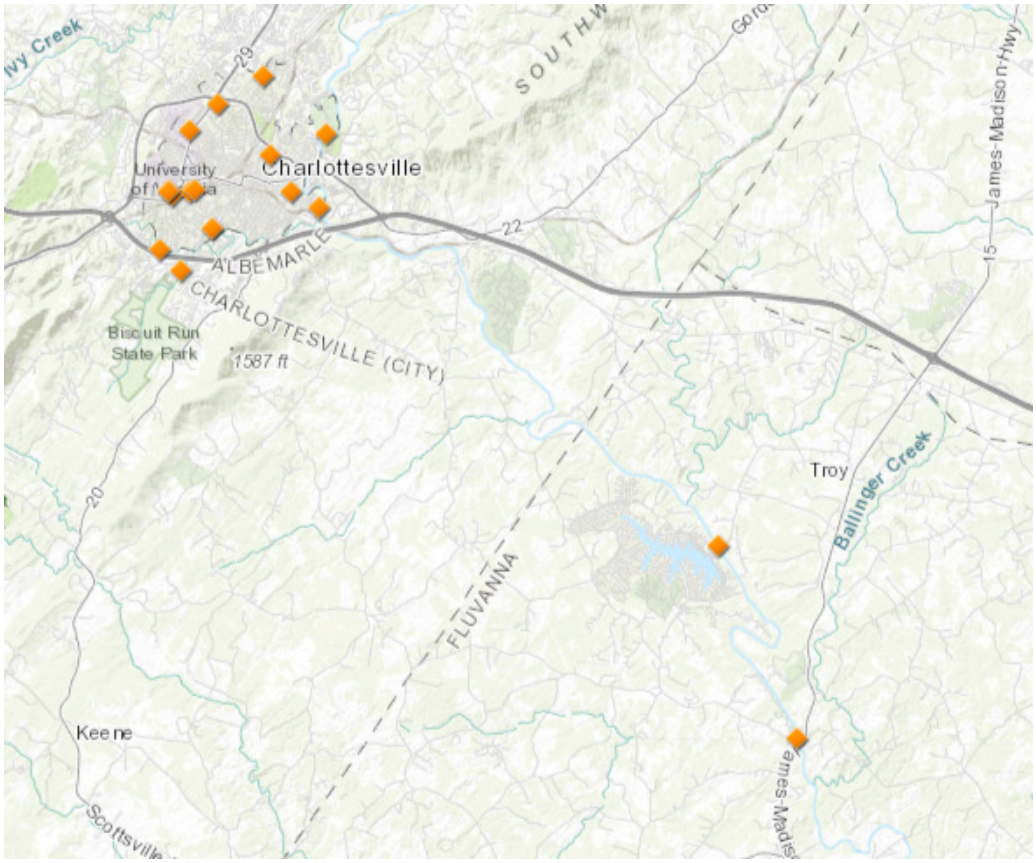
3. Sampling Technique

When sampling streams and rivers, it is critical to obtain a **representative sample**. This means that 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 pole to collect your water sample.

4. Sampling Time Frame

Volunteers will collect samples during daylight hours until 1:00 pm on the first Saturday of each month. Samples should be collected and **deposited to the lab by 1:00 pm** on Saturday to allow time for the Colilert sample to be run by the laboratory technician.

5. Sample Locations



Site Code	Site Name	Latitude	Longitude
BSC01	Biscuit Run West of Southern Pkwy	38.0037	-78.5087
LDC01	Lodge Creek @ 5th St SW	38.01674	-78.495829
LDC03	Lodge Creek near JPA	38.026649	-78.51153
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
RVN12	Rivanna River @ Palmyra	37.857763	-78.26679
RCK01	Rock Creek at Valley Road Extension	38.027889	-78.505065
RCK02	Rock Creek @ 5th St SW	38.016846	-78.495795
SCK01	Schenks Branch @250	38.041788	-78.475281
XLC01	Lodge Creek East Tributary	38.028302	-78.513082
XLD01	Lodge Creek West Tributary	38.028144	-78.513082
XRC01	Rock Creek Unnamed Trib - Patton St	38.028377	-78.503147

*For more detailed site information, please reference the **Bacteria Monitoring Site Guide**

6. 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 bottles, labeled with your site names
- Thermometer
- Data sheet(s) and clipboard
- Pencils & Sharpie
- Hard-sided cooler with loose ice (or ice in ziplock)
- Hand sanitizer

7. 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 pole is best for larger or deeper streams, provided the streambank is safe to sample from.

Sampling should take place in this order: 1) Bacteria 2) Temperature 3) Turbidity

Part 1- Bacteria Sample:

1. Walk upstream a short distance with minimal disturbance of the sediment.
2. Facing upstream, open the Coliscan bacteria sample bottle (**100 mL clear bottle with white cap**). Be sure to not contaminate the cap by touching the inside.
3. Carefully fill the bottle by submerging it below the surface as you move the bottle away from your body in an **upstream** direction using a "U" motion. This method ensures any bacteria from hands or boots does not enter the bottle. Avoid letting mud and other debris enter the sample bottle. When filling the Colilert bottle, fill between the 100 mL line on the bottle and the "shoulder" of the bottle.
4. Cap the bacteria sample bottle, dry it as needed with towel, and label it with the station ID and date using a waterproof pen or sharpie (for example: MWC 12 1/3/2019). 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 back to the lab.

Part 1a- Bacteria Sample with a "Dip Wand":

1. Affix bottle to end of sampling wand. Remove cap from bottle being careful not to touch the inside of the cap or the inside of the bottle.
2. Lower the wand carefully into the water. Using a U motion with the wand, fill the sample bottle by moving it through the water with the wand. When filling a Colilert bottle, fill between the 100 mL line on the bottle and the "shoulder" of the bottle.
3. Cap the bottle 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.
4. Fill out the Field & Lab Data Sheet with the necessary information.
5. Immediately place the sample bottle on ice for transport to the plating area.

Part 2- 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.

*use caution so that the tip of the thermometer does not scrape on rocks or sand in streambottom

Part 3- Turbidity Sample:

1. Use the bottle labeled "turbidity sample bottle" in your kit. This bottle gets reused each month.
2. Uncap the bottle and submerge the bottle below the water, allowing it to fill
3. Empty the bottle downstream of you.
4. Repeat the bottle submerging to fill the bottle for turbidity reading and cap the bottle.
5. Put it on ice in your pack.

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.
**If you are responsible for multiple sites, note total hours on one data sheet only.

8. Lab Procedures

After you have collected your sample, take it to the field lab located at 1150 River Road (the green cinder-block building).

A. Colilert Sample Delivery

1. Place your bacteria sample bottle labeled clearly with the Site Code and date in the refrigerator by 1:00 pm.

B. Turbidity Measurement

The turbidity meter lives in this case.

STEP 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.

STEP 2: Prior to calibrating the unit, wipe the "10 NTU" standard vial clean with a Kimwipe and place in the unit. Follow menu prompts and select "Measure", then "Turbidity – No Blank". Text is dark and the line is highlighted when it is selected.

1. Select "Scan Sample"
2. Press ENTER.

IF the readings are between 9.5 and 10.5, there is no need to calibrate the unit. If the reading is outside of this range, follow the **Calibration Procedures** at the end of this section.

STEP 3: Turbidity Measurement

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

2. Use the 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.

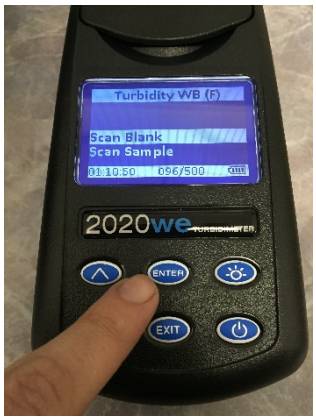
3. After the 3rd rinse, fill the vial to the white horizontal line.

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.

1. 

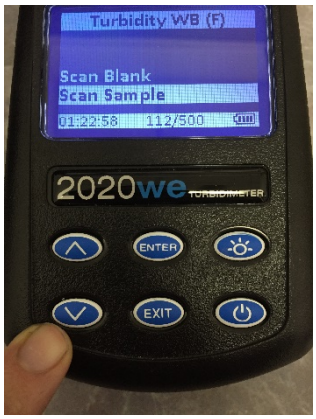
8. Select “Scan Blank”

9. Press ENTER.

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

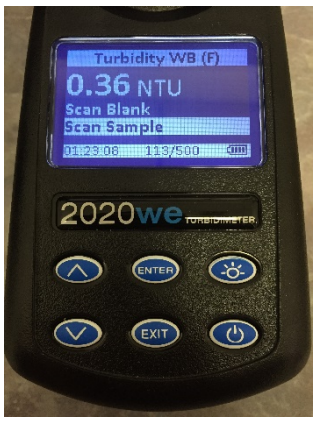
11. Insert the vial containing river water, again with white vertical line on vial positioned to face user.

12. Close turbidity meter cap

2. 

13. Select “Scan Sample”

14. Press ENTER.

3. 

15. Record reported turbidity value on the field sheet.

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

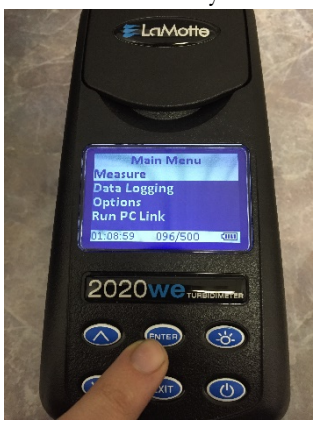
17. Turn the machine off and put everything back in its place.

18. Rinse your turbidity sample bottle back with tap water and leave next to sink to dry out.

C. Turbidity Meter Calibration

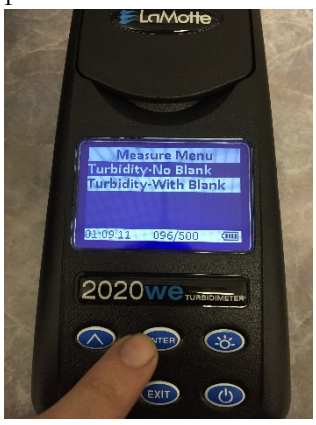
***Follow this procedure if unit not properly calibrated (see STEP 1)**

a. Turn on turbidity meter.

b. 

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

c. press ENTER

d. 

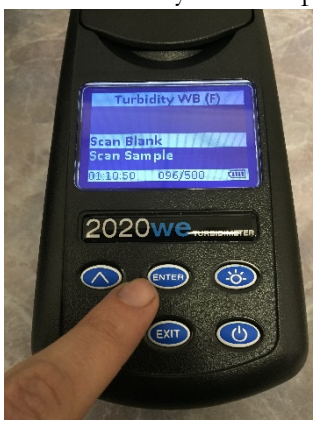
Select "Turbidity –With Blank",

e. Press ENTER.

f. 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.

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

h. Close turbidity meter cap.

i. 

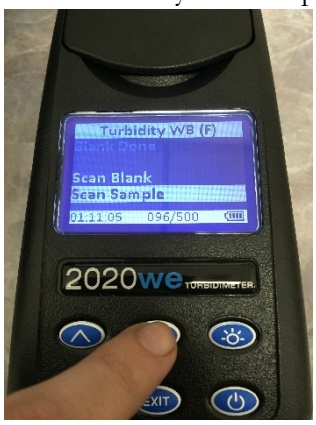
Select "Scan Blank."

j. Press ENTER.

k. After a few seconds, once unit has completed "blank" calibration, remove "blank" vial.

l. 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.

m. Close turbidity meter cap.

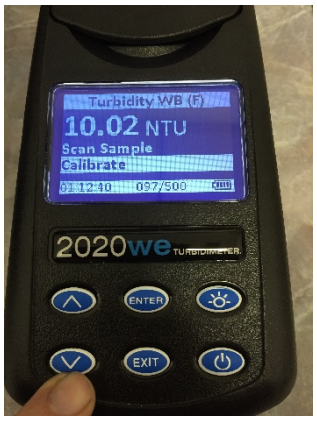
n. 

Select "Scan Sample"

o. Press ENTER.

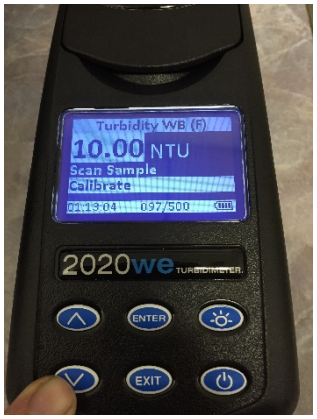
p. If the reported turbidity value equals the standard value of 10 NTU, calibration is complete and you may now continue to the steps below titled **Turbidity Measurement**.

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

r. 

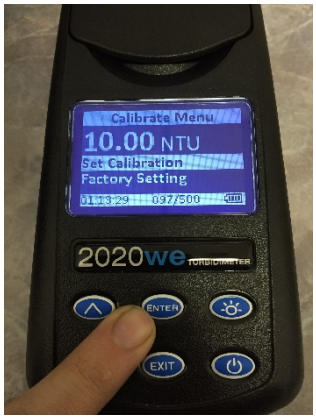
Press the down arrow key to highlight "Calibrate"

s. Press ENTER.

t. 

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

u. Press ENTER.

v. 

"Set Calibration" will be highlighted and you must

w. Press ENTER one more time to complete calibration.

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

9. Colilert Method for Laboratory Technician

Sample and Equipment Setup:

1. Confirm incubator is properly set at 35 $\pm$ 0.5 °C and record on the temperature log sheet.
2. Sterilize the bench surface where samples will be processed using 70% isopropyl alcohol or non-chlorinated disinfectant wipes.
3. Turn the Sealer on. After about 5 – 10 minutes it should warm up as indicated by the green light.
4. Remove samples from the sample cooler/refrigerator.
5. Confirm sample labels match information found on accompanying the Field & Lab Data Sheet.
6. Place a Quanti-tray in front of each bottle and label the paper backing with the station ID using a wide tip permanent marker, being careful not to puncture the paper.
7. Have Colilert media and unopened container if fecal bacteria free, non-buffered water ready (Deer Park is sufficient).
8. Fill out the laboratory analysis form with the required information.

Filling and Sealing Quanti-Tray:

1. Shake sample bottle to thoroughly mix the sample
2. Verify the sample bottle is filled to at least the 100 ml fill line and no more than the bottom shoulder of the bottle and remove the lid. Place the lid inner surface side up on the sterilized counter, being careful not to touch.
    a. If the bottle is overfilled, use a sterile pipette to remove excess water from the sample bottle so that the water level rests on top of the 100 ml line.
    b. If the bottle is underfilled, use a sterile pipette to add fecal bacteria free, non-buffered water to the sample bottle so that the water level rest on top of the 100 ml line.
        i. If using fecal bacteria free water, confirm the water used is free of bacteria by filling a sterile sample bottle to the 100 ml line and running a laboratory blank. Label this laboratory blank as LB
3. Shake or tap the Colilert media snap pack to ensure media collects to the bottom of the snap half of pack.
4. Snap open the Colilert media pack and pour contents of the pack into the sample bottle.
5. Recap the bottle and shake well until the media dissolves
6. Pick up the labeled Quanti-Tray associated with the sample. With the plastic well side facing the palm of the holding hand, gently squeeze the upper part of the tray so it bends towards the palm. Angle the tray approximately 60 to 75° with the foil side angled towards the ceiling.
7. Using the other hand, gently pull the foil tab to separate the foil back from the tray. **Do not** touch the inner surface of the foil or tray.
8. Pour the entire contents of the sample bottle with dissolved media into the tray opening. Tap the bottom of the tray to dislodge any air bubbles trapped in the wells.
9. Unbend the tray so the foil back is flush with the plastic well surface and place it in the orange 97 well tray carrier.
10. Place the tray carrier with the paper back side up onto the sealer tray loading rack. The small holes of the rubber carrier should be facing closest to the sealer.
11. Gently push tray towards the sealer until the sealer engages the tray carrier. **Do not** push the tray through the sealer
12. Place the used snap pack, sample bottle and pipette in the trash.

13. After the tray is sealed verify the wells are filled at least ¾ the way full. The large well at the top of the tray is for overflow and may not be ¾ full. If wells are not sufficiently full, note it on the associated Field & Lab Data Sheet or Lab Recording Sheet.
14. After processing no more than 10 trays, record the time the trays entered the incubator by writing on the paper back using a marker. Record times on the associated Field & Lab Data Sheet.
15. Place the sealed tray(s) in a 35°C ± 0.5°C incubator for 24 hours. Place trays plastic side down.
16. Wipe down sample preparation area with disinfectant and repeat steps 1-16 until all samples are processed.

Reading Trays After Incubation:

Safety Note: Never look directly at the UV light source when it is on or count samples on a reflective or glossy surface. Use UV blocking goggles or glasses to avoid eye damage.

1. Before retrieving samples, record incubation temperature on the temperature log sheet.
2. At 24 +/-2 hours remove sample trays from the incubator.
3. Count the number of Yellow wells and record the number of positive small and large wells on the associated Field & Lab Data Sheet. Yellow wells indicate coliform bacteria.
4. Turn the 325 nm UV lamp on. If necessary, turn off overhead lights or use a darkened light box.
5. Place the Quanti-Tray with the wells facing up under the UV lamp.
6. Wells that have a blueish glow under the UV and were yellow indicate *E. coli*. Record the number of small and large wells on the associated Field & Lab Data Sheet.

Use the IDEXX color comparators to verify questionable wells. Table 1 provides a narrative description of what to look for.

Table 1. Result Interpretation Table

Less yellow than the IDEXX comparator	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the IDEXX comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the IDEXX comparator	Positive for *E. coli*

7. Using the 97 well, Quanti-Tray 2000 Most Probable Number (MPN) table, record the Coliform and *E. coli* MPN on the associated Field & Lab Data Sheet.
    a. Confirm that the *E. coli* result is not higher than the coliform result. It can be equal but never greater than as *E. coli* is a member of the coliform family. If so, recount the associated tray.
8. After tray results have all been confirmed against datasheets, place in trash bag for disposal.
9. Wipe down preparation table with disinfectant.

Quality Control Procedures:

Equipment Blank:

At least one sample site per sample run will include a field blank. Field blanks are used to verify sample bottles and/or equipment used is free of contamination.

1. Label a sterile 100mL sample bottle with the Site ID that it is being collected at followed by "EB": e.g. Site 4 would be 4 EB.
2. In the comment section of the Field & Lab Data Sheet, record "Blank taken".

3. At the monitoring site, open the 100 ml sample bottle and container of sterile water. Do not touch the inner surface of either container or lid.
4. Pour the sterile water into the labeled field blank sample bottle and cap the bottle.
5. Place the sample in the Ziploc bag and cooler as a normal sample.
6. Transport and laboratory analysis of results are the same as outlined above.
7. Record results on the Field & Lab Data Sheet with the comment of "Field Blank"

Note: An acceptable field blank as <1 MPN *E. coli.* If *E. coli* results are 1.0 or greater, any samples collected by the sampler that day are flagged.

Field Replicate:

At least one sample site per sample run will include a field duplicate. A field replicate will be collected either at the same time or immediately after the primary sample is collected. If the sample is collected by bucket, the replicate uses the same water.

1. Label one sterile 100 ml bottle as normal and a second 100 ml sample bottle the Site ID that it is being collected at followed by "Rep": e.g. Site 4 would be 4 Rep.
2. On the comment section of the Field & Lab Data Sheet record "Replicate taken"
3. At the monitoring site, open the 100 ml sample bottle. Do not touch the inner surface of the bottle or lid.
4. Fill the replicate sample either at the same time or immediately after collecting the primary sample.
5. Place the capped replicate sample in the Ziploc bag and cooler as a normal sample.
6. Transport and laboratory analysis of results are the same as outlined above.
7. Record results on the Field & Lab Data Sheet with the comment of "Replicate"

Note: An acceptable replicate is when results of the samples are within 10x of each other (e.g. 200 vs 20 is acceptable, 200 vs 19 is not)

Laboratory Blank:

At least once per laboratory analysis run at after processing 20 samples, a laboratory blank is performed. This determines if there are contamination issues with the equipment or media used.

1. Label a sterile 100mL sample bottle as "Lab Blank".
2. Open a container of E. coli free, unbuffered water (such as Deer Park bottled water).
3. Fill the labeled sample bottle to the 100 ml line with the sterile water.
4. Follow the Quanti-Tray procedures outlined above.
5. Record results on the Field & Lab Data Sheet.

Note: An acceptable laboratory blank is <1 *E. coli* and <1 Coliform. If results are 1 or greater, the samples associated with the laboratory blank (up to 20 samples) are flagged.

Verification of Media Quality and Technician Analytical Technique:

Each lot of Colilert media received should be tested to ensure it is properly formulated. This test can also determine the analytical technique of laboratory personnel.

1. Remove the Quanti-Cult QC from the refrigerator and ensure the expiration dates are valid.
2. Remove one vial each of *E. coli*, *K pneumoniae*, and *P. aeruginosa* from the Quanti-Cult QC kit. Place the Quanti-Cult QC kit back in the refrigerator.

3. Prepare three 100 ml sample bottles and fill to the marked line with sterile, unbuffered water. Label each bottle and associated Quanti-Tray with the bacteria culture it will contain
4. Pour the tab from one vial into the corresponding 100 ml sample bottle containing sterile, buffered water.
5. Allow the tab to fully dissolve.
6. Follow the Colilert testing procedure outlined above.
7. Record results on the Field & Lab Data Sheet noting the results and that this is a Qunti-cult sample.

Note: Acceptable Quanti-Cult results are based on the lot number provided by the Quanti-Cult provider. If results are outside the acceptable range, the media may be bad or the technician may require retraining.

10. Coliscan Method (for Reference)

Step 1. Plate your bacteria sample

2. Plug in the incubator.
3. 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).
4. 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.
5. **Mix the water sample in the sample bottle by shaking or swirling for several seconds.**
6. 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.
7. **Gently swirl** the bottle of Coliscan media for several seconds so that it mixes with the sample water. **Do not shake the bottle** as this will cause the medium to foam and makes reading the colonies difficult during the counting phase.
8. 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.
9. 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.
10. Incubate the Petri dishes upside down for 24 hours at 35° - 40° Celsius. This is approximately 95° - 105° F.
11. 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.
12. Place your bacteria sample bottle labeled with the sample ID # and date in the refrigerator.

Step 2. Coliscan 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.

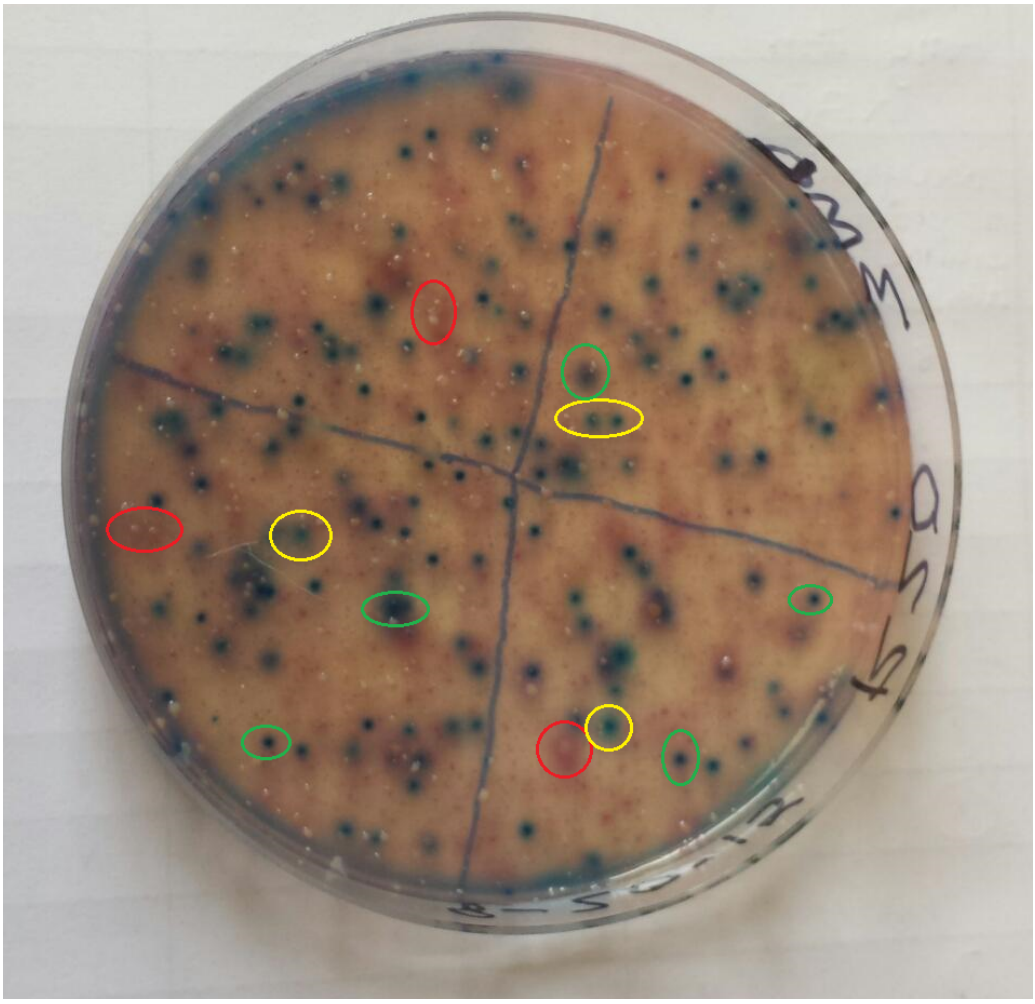
3. Count the number of **dark blue to royal purple** colonies on each plate and record this number in field sheet. Do not count teal colored or pink – dark red colonies or colonies colored anything other than **dark blue to royal purple**. If you are unsure of some colonies, you can use the microscope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

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

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.