

# Flint River GREEN: Fecal Coliform



<b>MATERIALS</b>	<ul style="list-style-type: none"> <li>• Coliscan Easygel (1 bottle/test)</li> <li>• Treated Petri Dish (1/test)</li> <li>• 1 to 5mL Test Tube/Sterile Pipette (<i>can use clean test tube from other test kit</i>)</li> <li>• Sterile Water Sample Collection Bottle</li> <li>• Cooler</li> <li>• Gloves (2/person)</li> <li>• Safety Goggles (1/person)</li> <li>• Incubator (or access to 48 hrs of 68°F)</li> </ul>	<b>VOCABULARY</b>	Total Coliform (TC) Fecal Coliform (FC) Effluent Coliform Bacteria <i>E. coli</i> Feces Pathogenic	Incubate Sanitary Sewer System Combined Sewer System Bioindicator
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<b>WATER QUALITY STANDARDS</b>	<u>DRINKING WATER (colony/100 mL water):</u> <1 <b>Total Coliform</b>	<u>SURFACE WATER (colonies/100mL water):</u> Total Body Contact (swimming): 200 <b>Fecal Coliform</b> Partial Body Contact (boating): 1000 Fecal Coliform Treated Sewage <b>Effluent</b> : No more than 200 Fecal Coliform
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<p><b>Coliform Bacteria</b> are a natural component of lakes, rivers, and streams. Total Coliform tests (referred to in drinking water standards) measure the total amount of bacteria in water from cold-blooded animals, warm-blooded animals, and soil organisms. Most bacteria found in streams are harmless to humans; however, some bacteria from the <b>feces</b> of warm-blooded animals <u>can</u> be <b>pathogenic</b> (such as some <i>e. Coli</i>). <b>Fecal Coliform</b> tests help us measure bacteria that only come from warm-blooded animals. Although this test combines both good and bad bacteria, high numbers of good bacteria often indicate high numbers of bad bacteria as well as other disease-causing organisms. The test works by mixing sample water with a prepared formula and <b>incubating</b> in a petri dish for up to 48 hours. Students then carefully count a.) purple colonies of bacteria that indicate fecal coliform and b.) pink AND purple colonies that indicate total coliform.</p>	<b>WHAT DOES THIS TEST MEASURE?</b>
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<b>LOOK FOR THESE CAUSES</b>	<u>EVIDENCE FOR DECREASES IN COLIFORM?</u> <ul style="list-style-type: none"> <li>• Do local residents regularly test and maintain any nearby septic systems?</li> <li>• Are nearby livestock kept away from streams?</li> <li>• Is there natural vegetation alongside streams (filters pollutants from run-off)?</li> <li>• Are manure and dog waste regularly removed or protected from rain and from surface run-off?</li> </ul>	<u>EVIDENCE FOR INCREASES IN COLIFORM?</u> <ul style="list-style-type: none"> <li>• <b>HUMAN</b>: Have there been <b>combined sewer system</b> or <b>sanitary sewer system</b> overflows? Possible illegal storm drain connections/dumping, leaking sewer pipes, failing septic systems? Are there marinas, campgrounds, or other waste pump-out facilities?</li> <li>• <b>NON-HUMAN</b>: What types of animals live nearby (wild/pets/farms)? Has there been recent flooding?</li> </ul>
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<ul style="list-style-type: none"> <li>• Unlike other water quality testing parameters, Fecal Coliform are living organisms, meaning they are a <b>bioindicator</b> of water quality. Many macroinvertebrates are also bioindicators of stream health.</li> <li>• Some contributing factors (such as manure and run-off) that increase coliform also affect other water quality parameters.</li> </ul>	<b>CONNECTING CONCEPTS</b>
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<b>WEB LINKS</b>	<ul style="list-style-type: none"> <li>• Technology Exploration— Wastewater Treatment Online Module: Michigan DEQ/Michigan Tech/MEECS: <a href="http://techalive.mtu.edu/meec/module21/title.htm">http://techalive.mtu.edu/meec/module21/title.htm</a></li> <li>• Case Study—USGS: Fecal-coliform Bacteria Concentrations in Streams of the Chattahoochee River National Recreation Area: <a href="https://pubs.usgs.gov/wri/wri004139/pdf/wrir00-4139.pdf">https://pubs.usgs.gov/wri/wri004139/pdf/wrir00-4139.pdf</a></li> <li>• Video—Plating Coliscan (8 min): <a href="https://www.youtube.com/watch?v=g1jj6J-rEII">https://www.youtube.com/watch?v=g1jj6J-rEII</a></li> <li>• Video—Incubating and Counting with Coliscan (8 min): <a href="https://www.youtube.com/watch?">https://www.youtube.com/watch?</a></li> <li>• Color Key for Counting Colonies: <a href="http://www.microbiologylabs.com/files/coliscan_easygel_color_guide.pdf">http://www.microbiologylabs.com/files/coliscan_easygel_color_guide.pdf</a></li> </ul>
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NOTE: *This test does not come packaged in a typical test kit.*

**While preparing for this test, decide if you will be plating the petri dishes while back at school OR at the stream. It is usually easier to do so back at school if there is time, otherwise you will need to carefully hold the dishes flat until the gel sets (45 min.).**

**A.) If plating back at school:** the collected water sample must immediately be placed on ice or in a cooler/refrigerator until used.

**B.) If plating at the stream:** be sure to keep the Easygel frozen until the day of the test, then keep it in a cooler to take to the stream until ready to use.

## PART 1: COLLECTION

1. Enter the stream with a sterile water collection bottle (an empty water bottle will work), wearing gloves and waders or boots. Aim to stand as close to the middle of the main stream flow as possible and try not to stir up any bottom sediment. SAFETY NOTE: Do not stand in water above your ankles if wearing boots or above your knees if you are wearing waders. DO NOT EVER enter flooded streams. Your group may find that collecting a large bucket of water from a bridge or by sending just one person into the stream may be safest for this as well as other tests.
2. Fill your collection bottle using standard water sample collection procedures. TIP: The test for Total Solids also requires a sterile water bottle, needs to be kept refrigerated, and requires at least 250 mL (a water bottle typically holds 500 mL), so you may decide to collect a full water bottle and use this sample water for both tests.
3. If you are doing the rest of the test back at school, cap the sample and place it in a cooler until you are ready to continue later. If you are plating back at school, you are now finished with the streamside portion of this test. When you are ready to plate, continue with **Step 4.**

## PART 2: PLATING

4. Check to be sure everyone is wearing gloves and goggles.
5. Inventory your supplies:
  - Treated petri dishes (1/test) - TIP: keep covered at all times except when pouring mixture and don't touch the inside of the dish
  - Coliscan Easygel medium (in cooler, 1/test)
  - Sterile Water Collection Bottle with Sample Water (in cooler)
  - 1–5 mL test tube/graduated cylinder/sterile pipette
6. Choose an amount of sample water to add to the Easygel bottle (between 1 and 5 mL) and carefully pour or pipette this amount into the bottle in a sterile manner. The amount doesn't matter as long as you remember how much you add. NOTE: If you are testing drinking water, always use 5mL.
7. Label the bottom (smaller, treated side) of your petri dish with the amount of water you used
8. Write down how much sample water you added to the Easygel bottle: \_\_\_\_\_ mL Water
9. Cap and swirl the bottle until the solution is completely mixed, then pour into the treated portion of a petri dish. Place the cover on your petri dish immediately and gently swirl until the entire bottom is covered.
10. Allow gel to solidify (30-45 min.) then flip dish upside down and incubate on a level surface following the directions below:
  - **Using an incubator:** 35°C (95°F) for **20–48 hours** (do not incubate beyond 48 hours)
  - **At Room Temperature:** 20–23°C (68-74°F) for **48 hours or more** (watch every 12 hours until some pink or purple colonies form, then wait an additional 24 hours to allow them to mature)

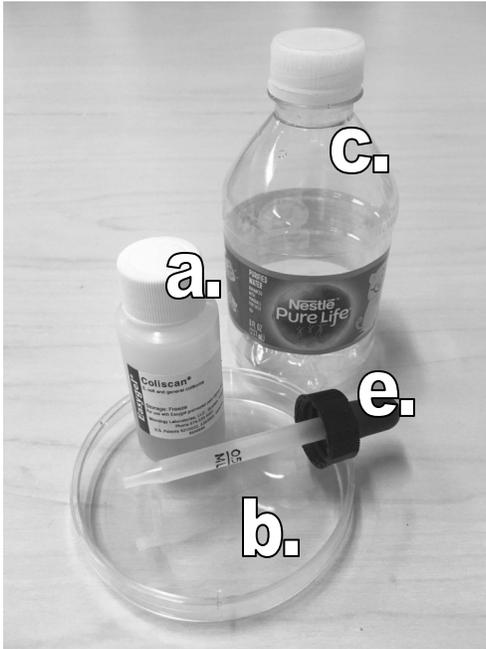
## PART 3: COUNTING COLONIES

11. Using the timeline from Step 10 in Part 2, determine when you should begin counting your pink (coliforms) and/or purple colonies (*E. coli* or fecal coliform). Other colored colonies of bacteria may grow such as white, blue or green—you may disregard these colonies because they are not coliforms. If you are doing stream testing, proceed to step 12. If you are doing drinking water testing, proceed to step 16.
12. **For stream testing**, count **only** the purple (fecal coliform) colonies in your dish. *TIP: You may wish to place your dish over a piece of graph paper to help you count.*
13. Record your result: \_\_\_\_\_ **fecal coliform colonies in dish**
14. Convert the number of colonies to report results in “Fecal coliform per 100 mL of water” by following these steps:
  - A. Divide 100 by the amount of sample water you added to the Easygel bottle in Step 6 (*you should have recorded this number on Step 7 and labeled your petri dish with this same number*):  $100 \div$  \_\_\_\_\_ mL (from step 7) = \_\_\_\_\_ **(multiplier)**
  - B. Use the multiplier result from above and multiply this by the number of fecal coliform colonies you counted in your petri dish:  
 \_\_\_\_\_ **(multiplier)** x \_\_\_\_\_ colonies = \_\_\_\_\_ **Fecal Coliform / 100 mL of water**  
 (For example, if you used 2.5 mL of sample water and counted 4 purple colonies of fecal coliform:  
 $100 \div 2.5 = 40$  and  $40 \times 4 = 160$  Fecal Coliform per 100 mL of water)
15. Any reading over 200 Fecal Coliform is cause for concern. Contact your mentor or the FRWC immediately. Unless you are testing drinking water, proceed to step 19.
16. **For drinking water testing**, you will need to calculate total coliform and not just fecal coliform. For total coliform, count all of the pink **AND** purple colonies (not green, blue or white). *TIP: You may wish to place your dish over a piece of graph paper to help you count.*
17. Record your result: \_\_\_\_\_ **total coliform colonies in dish**
18. Multiply the number of total coliforms by 20 (because you should have used 5 mL of sample water if you are testing drinking water and  $5 \times 20 = 100$ ) to convert to “total coliform per 100 mL of water”:  
 \_\_\_\_\_ **total coliform colonies in dish** x 20 = \_\_\_\_\_ **Total Coliform / 100 mL of water**
19. DO NOT dispose of your petri dishes or Easygel Bottles in the trash. Ask your teacher what they would like you to do with them (*Teachers: these can be disposed of safely once sealed in a Ziploc bag along with straight bleach or rubbing alcohol and mixed to ensure that all surfaces have been decontaminated*).
20. For stream testing, calculate a Q-Value on the Fecal Coliform Chart. **Q-Value:** \_\_\_\_\_
21. Check the Q-Value by entering your pH data at <http://www.flintrivergreen.org/add-info/add-data/>

## WHAT TO WATCH OUT FOR

- Always keep the Coliscan Easygel frozen until testing day. Keep in a cooler on testing day.
- If you transport the sample back to school for mixing and plating, be sure to use a cooler.
- If you plate the sample at the stream, be sure to keep it covered and very level until you get it back to school, then be sure to place it in the incubator or on a register right away.
- Make sure to check the sample at 12 and 24 hour increments.
- Make sure you use a treated petri dish and not a regular one.
- Make sure to pour the mixture into the treated side of the dish.
- Use sterile procedures (i.e. don't touch the inside) and introduce other bacteria to the sample.
- More detailed instructions: <https://www.microologylabs.com/page/95/Instructions>

Safety Data Sheet: [https://www.micrologylabs.com/files/25001\\_CS\\_Easygel\\_SDS.pdf](https://www.micrologylabs.com/files/25001_CS_Easygel_SDS.pdf)

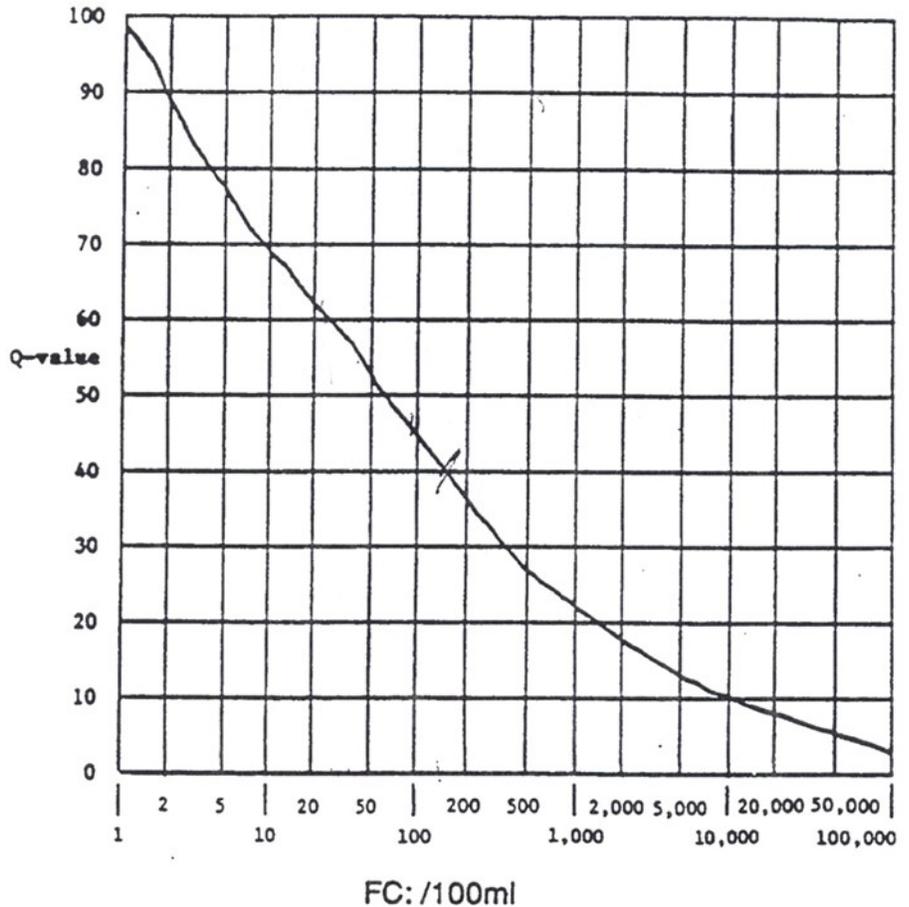


## Fecal Coliform Test

### Materials Needed to Perform the Test

- Coliscan® Easygel® Solution
- Easygel® Pretreated Petri Dish
- Collection Bottle (nonspecific)
- Pipet or Test Tube (must be able to measure 1-5mL of sample, can be taken from another test kit if is cleaned prior to use).

## Fecal Coliform Q-Value Chart



Note: if  $FC > 10^5$ ,  $Q = 2.0$