

FECAL COLIFORM MONITORING*

**These are teacher written directions.*

- **Note:** Preheat incubator to 44.5° centigrade. Sterilize the sample bottle, the top funnel of the filtration unit and the sample syringe in an autoclave or boil them for 5 minutes.
- **Sample for bacteria first to avoid stirring up the sediment at your sample location. It's important not to have any sediment in your bacteria sample.**
 1. Choose a safe sampling location in the stream, preferably where the water is flowing. Stand facing upstream to collect the water sample.
 2. Hold the sample bottle by the bottom and unscrew the bottle cap; hold the cap in one hand. Turn the bottle upside down and plunge it straight down into the water.
 3. When the bottle is completely submerged, tip the bottle opening up and into the current at about a 45 degree angle. Fill the bottle completely.
 4. Take the bottle out of the stream and pour off enough water to leave about one inch (1") of air space. Cap the bottle immediately. When you're back on shore, cover the top of the bottle with a piece of aluminum foil (this prevents any outside bacteria from sneaking into your sample). **If you are not running this test immediately, put your sample on ice in a cooler and deliver to appropriate location.**
 5. Add nutrient broth from one plastic ampule to a petri dish, put the lid back on and set aside.
 6. Sanitize forceps (tweezers) with alcohol.
 7. Unscrew the sterilized top half of the filtration system. Place a sterile filter paper on top of the filtration system's membrane with the sterile forceps (avoid contamination, do not touch with fingers!). Be sure to place paper grid side up with no wrinkles. See "Bottle Basics".
 8. Screw the top of the filtration system back onto the base.
 9. Using a sterile syringe, add 100 mL distilled water. Use the suction pump to draw the entire sample through the filter paper while swirling the unit to reduce the amount of bacteria adhering to the filtration unit's walls. Re-sanitize the forceps with alcohol. Unscrew the top half of the filter unit. Using the forceps, carefully move the filter paper into the petri dish, grid side up. Replace cover on dish. Label the petri dish with stream site and the amount of water filtered. Label this petri dish "control." Then repeat steps 5-16 three times with the sample water.
 10. Add a small amount of distilled water into the filtration unit through the top.
 11. Shake the bacteria sample bottle well. Using a sterile syringe, add the desired amount of the water sample into the filtration unit through top. (**Note:** If water quality at the sampling site is generally good, you may wish to filter an entire 100 mL sample. If water quality is poor, or if animals such as geese or cows are present, filter less water. If unsure, do three samples; 10 mL, 50 mL and 100 mL).
 12. Use the suction pump to draw the entire sample through the filter paper while swirling the unit to reduce the amount of bacteria adhering to the filtration unit's walls.
 13. Re-sanitize the forceps with alcohol. Unscrew the top half of the filter unit. Using the forceps, carefully move the filter paper into the petri dish, grid side up. Replace cover on dish.
 14. Label the petri dish with stream site and the amount of water filtered.
 15. Invert the dish (turn it up-side down) as you put in incubator. This allows any condensation to rise off of the lid, enabling you to see through the top of the dish. Incubate for 24 hours, plus or minus 2 hours.
 16. Count the colonies. **Remember to multiply by necessary factor.** For example, if you filtered 50 mL of river water, multiply by 2 to get the number of colonies per 100 mL

Fecal Coliform Data Sheet

Step #1: Fill out all the information below.

School: _____	Weather: _____
Teacher: _____	Air Temperature: _____
Names of Monitors: _____	Test Kit: (Hach, LaMotte or other) _____
Stream Name: _____	Date: _____
Test Location: _____	Time: _____

Step #2: Record at least 3 replicate sample values in the chart below. Remember to run a blank sample plate (using just distilled water) to check for equipment contamination.

Replicate #1	Replicate #2	Replicate #3	Replicate #4
_____ FC/100mL	_____ FC/100mL	_____ FC/100mL	_____ FC/100mL

Step #3: Record the **highest fecal coliform values** of your 3 replicate samples in the box below. Record any comments or observations.

Test Result (record the highest)	_____ FC/100mL	Comments: _____ _____ _____
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Step #4: Record two fecal coliform test results from previously recorded data for your site in table below.

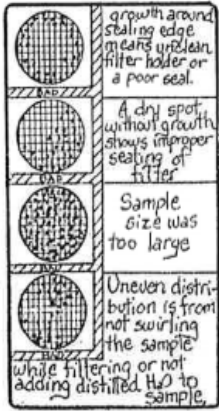
Step #5: Record comments from your comparison.

Test Results Date: _____	_____ FC/100mL	Comments: _____ _____ _____
Test Results Date: _____	_____ FC/100mL	_____ _____

Step #6: Have the recorder sign once each step is complete.

Test Completed _____
 Date: _____
 Data Reviewed _____
 Date: _____
 Data Transferred to Master Data Sheet _____
 Date: _____

Optimal Fecal Coliform Values: For optimal values for salmon, fecal coliform values should not exceed 100 FC/100mL on average.
 However, less than 50 FC/100ml is optimal



Troubleshooting fecal coliform cultures.