Fecal Coliform

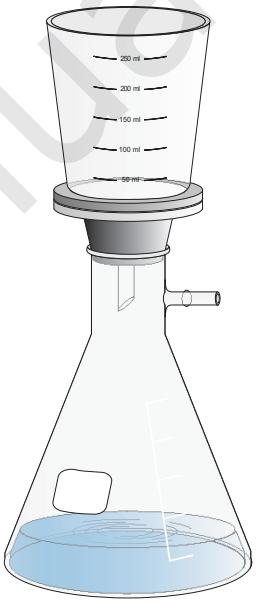
INTRODUCTION

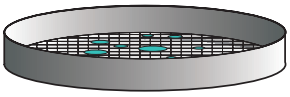
The concentration of fecal coliform bacteria in water is measured to determine the likelihood of contamination by microbiological organisms. While fecal coliform are not pathogenic (disease causing), they are commonly found alongside pathogenic organisms such as those responsible for dysentery, gastroenteritis, and hepatitis A. It is easier to test for fecal coliform than for pathogenic organisms; therefore, the presence of fecal coliform in a water sample is used to indicate potential contamination. A common source of coliforms and pathogenic bacteria is raw sewage. Fecal coliform bacteria occur naturally in the digestive tract of warm-blooded animals, where they aid in the digestion of food.

Sources of Raw Sewage

- Urban stormwater runoff containing domestic animal waste
- Agricultural sources such as dairies and cattle
- Sewage treatment overflow

The results of coliform bacteria tests are generally used to monitor recreational areas, stormwater out-falls, and drinking water supplies. Water is commonly tested for three types of coliform bacteria: fecal coliforms, total coliforms, and *E. coli*. The standards for drinking water are generally based on total coliforms. The accepted standard for drinking water is that there should be no coliforms present after the water is filtered or treated. Natural waters will nearly always contain some form of bacteria. That is why you should never drink untreated water from a river or lake. Currently, the most common measurement for surface waters is fecal coliform. In some areas, standards for surface water contamination are shifting to the measurement of *E. coli*.





Expected Levels

Standards for fecal coliform differ from state to state. For specific requirements, it is best to contact your state or regional health department. Standards for fecal coliform are considerably more strict if the water is used for total body contact such as swimming, rather than used only for boating with minimal direct contact.

When interpreting data from fecal coliform tests, it is important to remember that there can be a high degree of randomness of distribution within a sample. A large number of data points are necessary to obtain statistically significant data. Fecal coliform is measured in *colony forming units* per 100 mL, CFU/100 mL, of water tested.

Table 1[1]

Water use	Desired level (CFU/100mL)	Permissible level (CFU/100mL)
drinking	0	0
swimming	<200	<1,000
boating or fishing	<1,000	<5,000

Summary of Method

This test uses the membrane filtration technique to measure quantity of fecal coliform bacteria present in a water sample. Samples are obtained from a water source, such as a river or stream, using Whirlpak containers. Water is then filtered through sterile 47 mm filters. The filters are placed in petri dishes containing mFC broth and incubated for a period of 24 hours at 44.5°C. Following incubation, the petri dishes are removed from the incubator and each dish is counted for fecal coliform colonies.

[1] CFU values in this chart were obtained from the LaMotte Company's "*The Monitor Handbook.*" These values are meant to be used as guidelines. Consult your local or state health department or your regional USEPA or USGS office for specific values for your region.

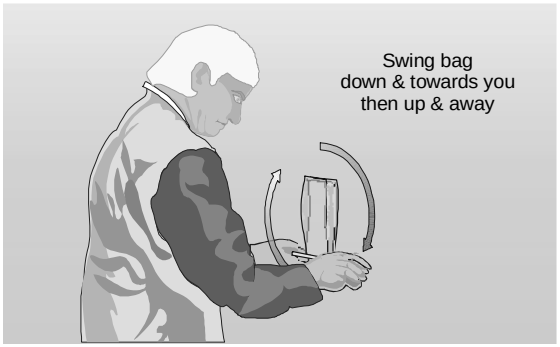
FECAL COLIFORM

Materials Checklist

___ 200 mL Whirlpak bags, sterile
___ magnetic filtration holder, sterile
___ 1000 mL filtering flask
___ hand vacuum pump
___ 8 sterile 0.45 µm membrane filters
___ 8 mFC broth/Rosolic acid ampoules
___ 8 sterile petri dishes with absorbent pads
___ 4 sterile, capped tubes, 10 mL
___ autoclave
___ ethanol
___ 400 mL beaker
___ alcohol burner or other flame source
___ sterile pipettes (1 mL and 10 mL)
___ sterile disposable plastic pipettes
___ sterile 10 or 50 mL graduated cylinder
___ sterile water
___ waterproof marker
___ small metal forceps

Collection of Water Samples

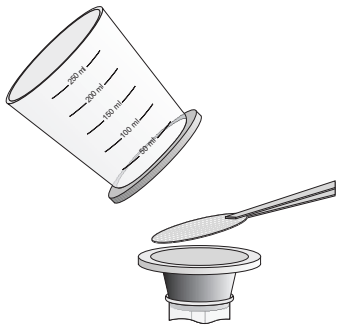
1. Use a waterproof marker to label a Whirlpak bag with the site code, date, and time of collection.
2. Collect one 200 mL Whirlpak of sample water, using sterile technique.
   a. Open the top of the Whirlpak along the perforated line.
   b. Pull the mouth of the Whirlpak open using the yellow pull tabs. Make sure you do not touch the inside of the bag—it is pre-sterilized.
   c. While holding the bag by the pull tabs, immerse the Whirlpak in the flowing portion of the stream. Make sure you are standing downstream from the sample.
   d. With the mouth of the Whirlpak facing into the current, the water pressure will force the Whirlpak open as the water flows in and fills it.
   e. Carefully remove the Whirlpak from the stream.
   f. Stand so you are not facing anyone. Hold the Whirlpak with one tie in each hand.
   g. Twirl the Whirlpak several times, with the bottom swinging towards you, then up and away. Once the excess air is pushed out, the Whirlpak will be pulled tight.



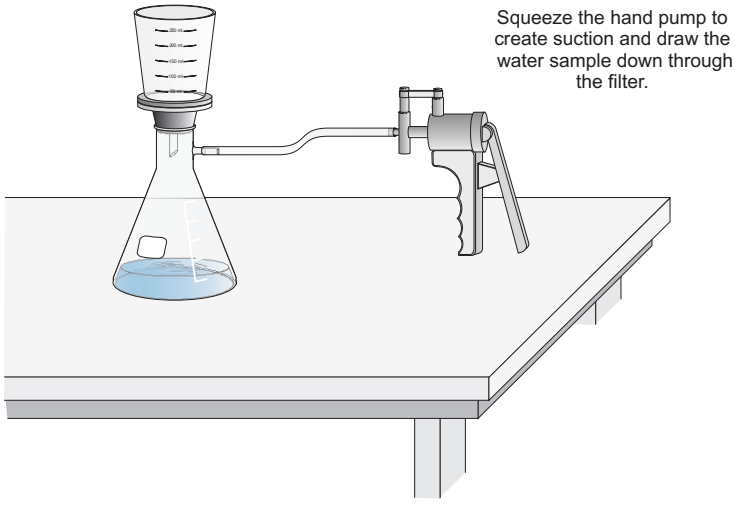
   h. Twist the ties together to close the Whirlpak.
3. If filtering in the field, you should filter the sample within one hour of the time it was collected. Otherwise, place the sample in an ice chest for transport back to the lab. Once you have returned to the lab, refrigerate the sample and filter within 24 hours.

Filtration Technique

1. Obtain eight 47 mm sterile petri dishes from your instructor.
2. Label each petri dish with the site code, date, and volume of sample being filtered (0, 1, 10, 30 mL). For each site water was collected at, there will be two petri dishes for each sample volume, as follows:
   - Two 10 mL water blanks (0-mL control)
   - Two 1 mL samples (diluted in a 10-mL sterile water blank)
   - Two 10 mL samples
   - Two 30 mL samples
3. Using the ampoule breaker, open and pour 1 ampoule of mFC/Rosolic Acid Broth into each of the petri dishes. Place the lids back on each of the petri dishes and discard the empty ampoules.
4. Remove the top of the filtration unit. Using sterilized forceps, carefully place a membrane filter onto the filter holder and pour a small amount of sterile water onto the filter to help seat it.



5. Filter the samples in the order they are written in Step 2, beginning with the 10 mL blank. Pour your sample into the apparatus. Be careful to avoid pouring the sample on the inside walls of the filtration apparatus. Suction the sample through the filter by squeezing the hand pump a couple of times to create a vacuum. Make sure most of your sample flows through the filter and does not remain inside the filtration apparatus. When all the water is through the filter, stop pumping and break the vacuum.



6. Rinse the filtration unit with three separate 30 mL portions of sterile deionized water.

7. Sterilize the forceps by dipping them into a bottle of ethanol (or other alcohol) and passing them through a flame. Allow the forceps to cool briefly before touching anything.

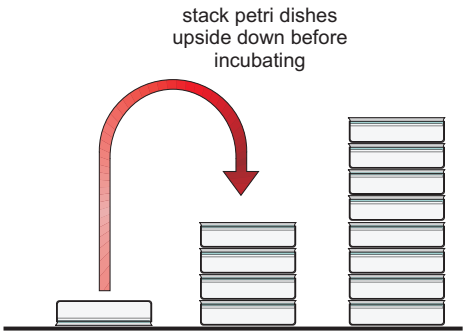
8. Carefully remove the filter with flame-sterilized forceps and place the filter gridside up on the agar in the proper petri dish. If there are air pockets between the filter and agar, carefully tap the filter down with the forceps.

9. Place the lid on the petri dish. Keep each petri dish upright until you are ready to incubate all of them.

10. Repeat Steps 4–9 for the remaining samples.

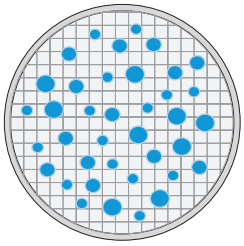
11. Stack the petri dishes upside down and incubate them for 24 hours in a controlled 44.5°C incubator. If no incubator is available, place the dishes in a plastic bag and seal it shut with water-proof tape. Place the bag, so that the dishes are upside down, in a water bath set to 44.5°C for 24 hours. **Important:** The petri dishes must be stacked upside down before they are incubated.

12. Make sure you sterilize the entire setup before filtering water from another site.



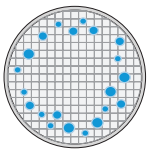
Calculating Results

1. When the petri dishes have incubated for 24 hours, remove them from the incubator and count the fecal coliform colonies that appear on each filter. Each bluish spot should be counted as one fecal coliform colony. Cream-, gray-colored, and colonies of other colors are not fecal coliform colonies. Do not include them in the count.



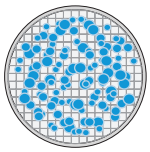
correct when you have 20-80
evenly dispersed colonies

2. If the petri dish has colonies that are more numerous than 80, fewer than 20, or unevenly distributed, there might be a problem with the sample. This chart details possible problems you might experience and what the cause is likely to be. Petri dishes that fall into this category should not be included in your count.



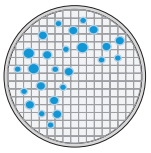
Result: growth around edge only.

Problem: unclean filter holder or poor seal.



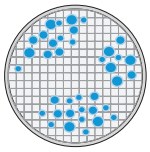
Result: colonies too numerous to count.

Problem: sample size too large.



Result: dry spot with no growth.

Problem: improper seating of filter.



Result: colony distribution is uneven.

Problem: sample was not swirled while filtering.

3. For each sample size (mL filtered), record the number of colonies counted on the Data and Calculations sheet. If a single filter has more than 200 colonies, record that filter as Too Numerous To Count (TNTC).

4. After all petri dishes have been counted and recorded, use a checkmark to indicate which sample volume is being used for the final calculation, based on the following rules:

   a. Most accurate counts are in the range of 20–80 colonies per filter. Use the average of all filters falling in that range. Calculate the Colony Forming Units (CFU) per 100 mL using the formula

$$\frac{\text{average colony counts}}{\text{mL filtered}} \times 100 = \text{CFU/100 mL}$$

   b. If there are no filters with 20-80 colonies, but there are filters of more than 80 colonies that are still clearly "countable," use the >80 colonies sample. Calculate CFU/100 mL based on the same formula as above. With higher counts and less defined colonies, competition can produce significant errors in coliform counts.

   c. If all countable filters have fewer than 20 colonies, estimate the CFU/100 mL using all filters. Add up the total number of counts and the total number of mL filtered (include ∞ colony petri dishes and volumes). Use the formula below and report the results as an estimate.

$$\frac{\text{Total colony counts}}{\text{Total mL filtered}} \times 100 = \text{CFU/100 mL}$$

   d. If you have conflicting colony counts for different sample sizes, calculate the CFU/ 100 mL using the smaller sample size only. For example, if you count 37 colonies in a 10 mL sample and 41 colonies in a 30 mL sample, use only the 10 mL sample size.

5. Record the calculated CFU/100 mL on the Data & Calculations sheet and use this value for comparing the water quality between different sites.

DATA & CALCULATIONS

Fecal Coliform

Stream or lake: _____ Time of day: _____

Site name: _____ Student name: _____

Site number: _____ Student name: _____

Date: _____ Student name: _____

	A	B		C	D
(√)	mL filtered	Number of colonies Filter 1	Filter 2	Average	CFU/100 mL

E	Final Value	

Column Procedure:

A. Record the volume of sample water filtered.
B. Record the number of colonies counted from each filter.
C. Calculate the average colonies counted from both filters.
D. Calculate the CFU/100 mL. (See the Calculating Results section on Page 9 - 5.)
E. Select the most representative sample and record the CFU/100 mL as the final value for this site.

Field Observations (e.g., weather, stream flow, pollution sources along stream) _____

_____

_____

Test Completed: _____ Date: _____

Vernier Lab Safety Instructions Disclaimer

THIS IS AN EVALUATION COPY OF THE VERNIER STUDENT LAB.

This copy does not include:

- **Safety information**
- **Essential instructor background information**
- **Directions for preparing solutions**
- **Important tips for successfully doing these labs**

The complete *Water Quality with Vernier* lab manual includes 16 water quality tests and essential teacher information. The full lab book is available for purchase at: http://www.vernier.com/cmat/wqv.html

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