Water Quality Analysis

Objectives:

1. Determine if *E. coli* or other coliform bacteria are present in a sample of water.
2. Determine if the water sample is potable using standards for water quality.
3. Describe the membrane filter technique used to determine potable water quality.
4. Explain the advantages and disadvantages of the membrane filter technique.

Key Terms

Coliforms, potable, membrane filter

Introduction

Drinking water is usually obtained from groundwater, rivers, streams, and reservoirs. All water sources contain bacteria. Most of those bacteria are harmless. Some have the potential to cause disease. Coliforms are Gram-negative rods that do not produce spores and can ferment lactose. However, because not all coliforms actually ferment lactose, the definition of a coliform has been expanded to include Gram-negative rods that produce the enzyme β-galactosidase. This enzyme cleaves lactose into its component monosaccharides: glucose and galactose. Fecal coliforms are found in the intestines of homeothermic (warm-blooded) animals. Coliforms found in feces include bacteria such as *E. coli* and *Enterobacter sp*. which are not particularly harmful. Of concern is the presence of potential pathogens in feces such as *Salmonella* and *Shigella.* If feces is contaminating the water supply, these pathogens will be present. However, if the sick individual recovers, the pathogens will no longer be excreted and they will disappear from the water supply. If fecal contamination is an ongoing problem, then fecal coliforms will be present. It is easy to test for the presence of fecal coliforms. Therefore, fecal coliforms are used as indicators of water quality.

The recommended standard by the U.S. Public Health Service for drinking water is no more than 1 coliform per 100 mL. The recommended total bacterial count (non-coliform) is no more than100 bacteria/mL.

The advantages of the filter method as compared to other methods are that it is relatively fast. Results can be observed in 24 hours. The filter method is sensitive. Large volumes of water can be tested which would find relatively few contaminants. The filter method is accurate and quantitative. Colonies can be counted and a concentration can be calculated. There are also some disadvantages. The water sample must be relatively clear. Turbidity will clog the filter. Highly contaminated water

must be diluted in order to obtain discrete, countable colonies. The procedure would have to be repeated and more supplies expended.

The m-ColiBlue system is used to determine the presence of coliform bacteria in water samples. When the filtered samples are incubated, *E.coli* forms blue colonies. Other coliforms will show as red colonies. All other colors are non-coliform bacteria.

Materials

Live Organisms: *possible coliforms*

Pond water sample, aquarium water sample, Membrane filter apparatus, M-Coli blue medium, forceps, alcohol for flaming, 1 mL pipettes and pipettors.

Per Table: Each table has a beaker of pond water and aquarium water.

Procedure

1. The sterile membrane filter container is already attached to the vacuum manifold-leave in place.

2. Each group will test one of the following water samples: pond water, aquarium water, or a sample brought in by a member of the group.

3. Obtain a water sample. If using pond water, add 1.0 mL to a 99 mL sterile water bottle. If using aquarium water, add 1.0 mL to a 99 mL sterile water bottle. Note: Instructor may change the amounts of each sample to add depending on environmental conditions. If using tap water as your water sample, then add 100mL.

4. Carefully remove the lid from the filter container. If the container comes off of the vacuum manifold, reattach it making sure that it is firmly seated in the manifold.

5. Pour 100 mL diluted water sample into the filter container. Put the lid back on. The lid should be askew and very loose. The entire class will be filtering at the same time.

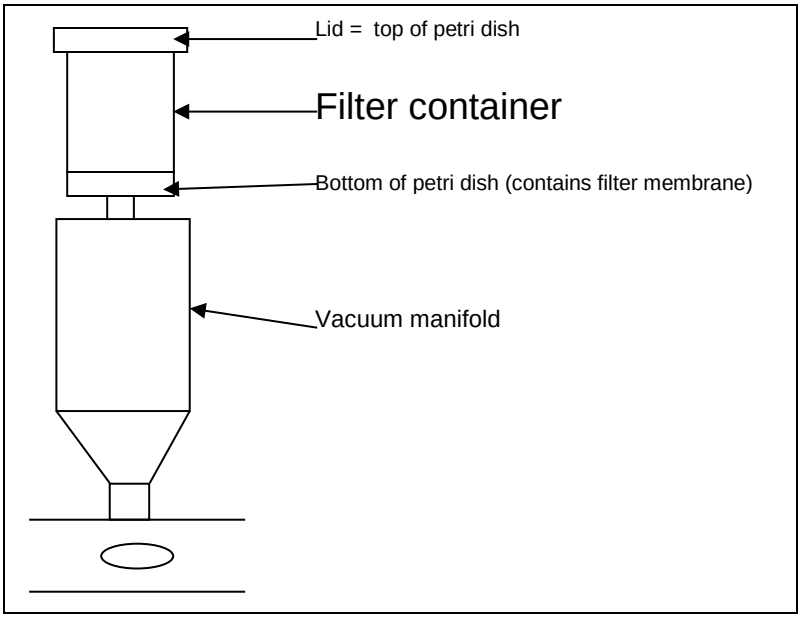
6. After the water sample has been filtered, put the lid securely back on the filter container, remove container and put a blue cap on the bottom of the container plugging the hole.

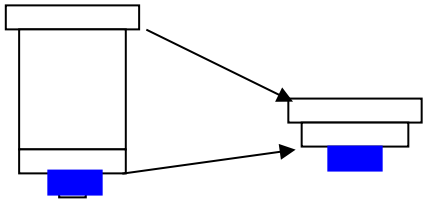
7. Separate the container bottom (has membrane with bacteria stuck on it) and the lid from the rest of the apparatus. Attach the lid to the bottom to make a “petri dish”.

8. Use sterile forceps to lift the filter membrane off of the absorbent pad. Carefully squeeze the m-ColiBlue broth ampoule and saturate the absorbent pad with broth.

9. Lay the filter membrane back on top of the absorbent pad. Make sure filter is flat on the pad without any bubbles. Close the little petri dish.

10. Label the dish with your group name and the sample used. Incubate upside down at 37ºC.





Safety and Disposal

1. Return the 99 mL sterile water bottles to side counter for recycling.

2. Discard the plastic filter “body” into the trash.

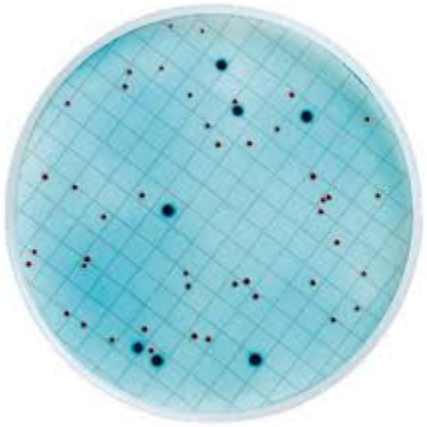
3. Discard pipettes into containers on tables.

4. Aprons must be worn. Hair must be tied back and shoes must cover the entire foot.

Observations

Count the number of blue and red colonies. The ideal range is 20-80 colonies but count whatever you have.

Your individual group data.



Number of Colonies		
	Pond	**Aquarium**
Blue		
Red		
Total		

Divide the total number of colonies (blue + red) by the mL of sample and multiply by 100.

$$\frac{\text{\# colonies}}{\text{mL sample}} \times 100 = \text{number of coliforms per 100 mL}$$

Class Data

Coliforms per 100 mL		
	Pond	**Aquarium**
Table 1		
Table 2		
Table 3		

Concept Check

1. Based on your results, is your water sample potable?
2. How do you recognize coliforms on the m-ColiBlue petri plates? *E. coli* specifically?
3. Why are we concerned about coliforms in drinking water?
4. Why are coliforms used as indicator organisms for water quality?
5. Describe the advantages and disadvantages of using the membrane filter.
6. Describe coliform bacteria.