Microbiology Lab Experiment Changes

Experiment #: 7-5

Title: Quantitative Analysis of Water: Membrane Filter

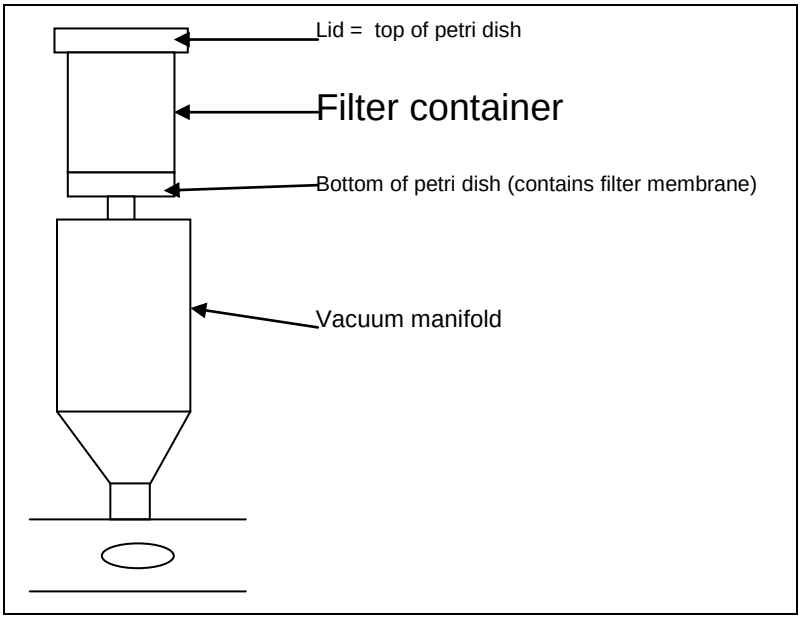
Live Organisms: putative *E. coli*

Changes: Procedure (Work in groups)

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.

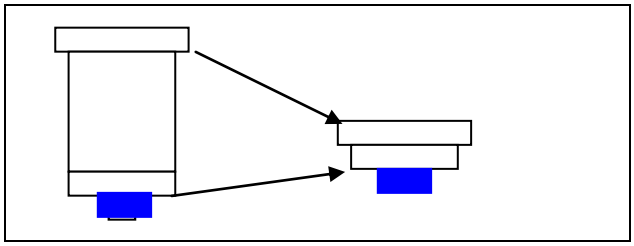


4. Carefully remove the lid from the filter container. If the container comes off of the vacuum manifold, reattach it making sure that it is firmly seated in the manifold.

5. Pour 100 mL diluted water sample into the filter container. **Don't put lid back on until water has been filtered thru the membrane.** The entire class will be filtering at the same time.

6. After water sample has been filtered, put the lid 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 also separate the lid. Attach the lid to the bottom to make a "petri dish".



8. Add enough Endo broth to saturate the membrane. Discard excess Endo broth in the trash.

9. Label plate and incubate topside up.

Next Lab Period:

1. Be careful opening plates (may have excess liquid). Observe the types of colonies by color:
   - shiny green/gold colonies = *E. coli*
   - dark red/purple colonies = coliforms
   - any other colors = non-coliforms

2. Count the number of dark red-purple colonies (with or without green/golden sheen). The ideal range is 20-80 colonies but count whatever you have.

Divide the number of colonies counted by the mL of sample and multiply by 100.

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

Take Home Lesson: Coliform bacteria are gram negative, non-spore forming, facultatively anaerobic rods that ferment lactose. They are enterics and are used as indicator organisms for the possible presence other enteric pathogens such as *Salmonella* and *Shigella* also found in feces. *E. coli* is the most commonly used indicator organism.

Based on your results, is the water potable? 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 is no more than100 bacteria/mL.

Advantages of the filter method as compared to other methods:
- relatively fast (results in 24 hrs)
- large volumes of water can be tested which would find relatively few contaminants (sensitive)
- accuracy, each colony represents a clone of a single bacterium; results reproducible and quantitative.

Disadvantages: turbidity clogs filter, highly contaminated water must be diluted in order to obtain discrete, countable colonies.