

## 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. Obtain a complete sterile membrane filter assembly:
  - filter apparatus
  - sterile membrane
  - syringe
  - jar of alcohol and forceps
  - little petri dish with pad
  - vial of Endo broth
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. Remove the apparatus from the sterile bag. Loosen the upper reservoir by unscrewing it but do not unscrew completely.
4. Dip forceps in alcohol and flame them. Carefully remove the filter membrane from its package. **Note: The filter membrane is sandwiched between two pieces of paper. Remove the filter membrane with the forceps being careful not to touch membrane with fingers.**
5. Remove the upper reservoir and place the membrane grid side up on the filter holder (grille) between the upper and lower reservoirs.
6. Screw on upper reservoir and attach syringe to lower reservoir.
7. 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. Remove filter apparatus lid and add the diluted pond or aquarium water to the upper reservoir. Replace lid. If using an alternate water sample you brought, then add 250mL. The upper reservoir is graduated.
8. Draw the water thru the filter by pulling and pushing the syringe plunger back and forth. This creates a vacuum in the lower reservoir.
9. In the meantime, obtain the petri pad dish and the ampoule of Endo broth. Carefully snap the neck of the ampoule and apply the Endo to the petri pad.
10. When the entire sample has been pulled thru the filter, flame forceps and remove the membrane filter from the apparatus and place in a petri dish on top of the saturated pad. Close petri dish and label. Incubate at 37°C.

### Next Lab Period:

1. Observe the types of colonies by color: red, purple (reddish purple), shiny green or golden.
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:** Based on your results, is the water potable? Advantages of the filter method as compared to the lactose tube method: 1) takes less time, no confirmed or completed tests to perform; 2) large volumes of water can be tested which would find relatively few contaminants; 3) accuracy, each colony represents a clone of a single bacterium, results reproducible. Disadvantages: turbidity clogs filter, highly contaminated water must be diluted in order to obtain discrete, countable colonies.

Describe the filter apparatus and know the purpose of each part.