Bacteriologic Water Quality: Membrane-Filtration*

OBJECTIVES

After completing this exercise, you should be able to:

1. Explain the principle of the membrane filter technique.
2. Use the membrane filter technique to determine the bacteriologic quality of water.

BACKGROUND

Fecal contamination of water can be determined by the presence of fecal coliforms or enterococci in a water sample, by the multiple-tube technique. The bacteria can also be detected by the membrane filter technique.

In the **membrane filter technique,** water is drawn through a thin filter. Filters with a variety of pore sizes are available. Pores of 0.45 µm are used for filtering out most bacteria. Bacteria are retained on the filter, which is then placed on a suitable nutrient medium. In field situations, nutrients are added to a thick absorbent pad on which the filter is placed. Nutrients that diffuse through the filter can be metabolized by bacteria trapped on the filter. Each bacterium that is trapped on the filter will develop into a colony. Bacterial colonies growing on the medium can then be counted. When a selective or differential medium is used, desired colonies will have a distinctive appearance.

Selective Media

Endo agar is frequently used as a selective and differential medium with the membrane filter technique. The composition of Endo agar is shown in Table 1. Endo agar is selective because desoxycholate and sodium lauryl sulfate inhibit gram-positive bacteria. Endo agar is differential in that the colonies of lactose-fermenting bacteria have pink to red colonies with a metallic green sheen (Figure 1).

The enterococci are fecal streptococci that include *Enterococcus faecalis* and *E. faecium*. *Enterococcus* spp. differ from streptococci by their ability to grow in 6.5% NaCI, at pH 9.6, and at 10°C and 45°C. **Enterococcus agar** is a selective and differential medium for enterococci (Table 2). Azide inhibits growth of gram-negative bacteria, and the dye TTC is picked up by growing cells to produce red colonies (Figure 2).

Table 1. Endo Agar	
Tryptose	0.75%
Yeast extract	0.12%
Lactose	0.94%
Dipotassium phosphate	0.4%
Sodium desoxycholate	0.01%
Sodium lauryl sulfate	0.005%
Basic fuchsin	0.008%
Agar	1.5%
Source: *Difco Manual.*	

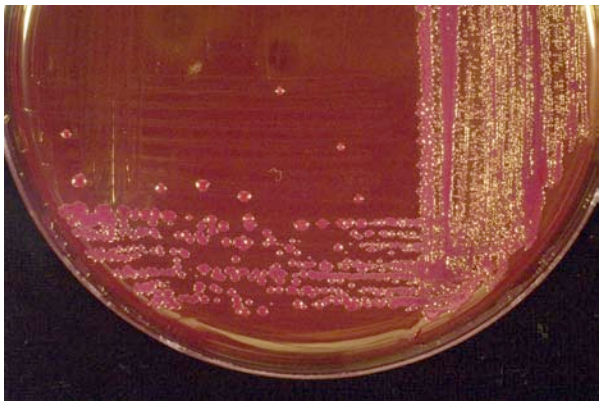

Figure 1. Endo agar. Coliforms produce rose-red colonies with a metallic sheen within 24 hours.

Table 2. Enterococcus Agar	
Tryptose	2.0%
Yeast extract	0.5%
Dextrose	0.2%
Dipotassium phosphate	0.4%
Sodium azide	0.04%
Agar	1.0%
Triphenyl tetrazolium chloride (TTC)	0.01%
Source: *Difco Manual.*	

* Johnson, T. and C. Case. 2010. *Laboratory Experiments in Microbiology*. San Francisco: Benjamin Cummings.

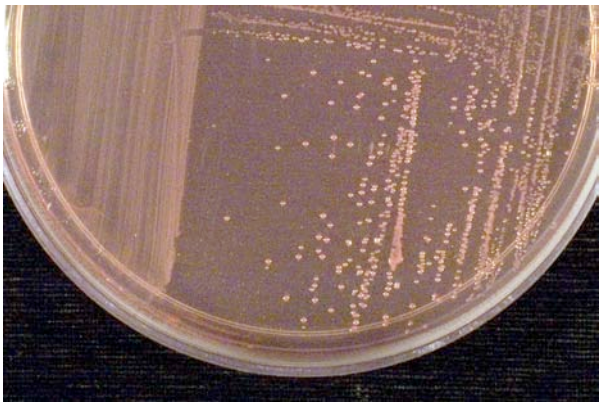

Figure 2. Enterococcus agar. Enterococci produce pink to red colonies; gram-negative bacteria are inhibited by azide in the agar.

MATERIALS per 3 students

FIRST PERIOD

Water sample or bring your own from a pond or stream
47-mm Petri plate containing Endo Agar
47-mm Petri plate containing Enterococcus agar
Sterile membrane filter apparatus
Sterile 0.45-µm filters (2)
Blunt-tip forceps
Alcohol
Sterile pipette or graduated cylinder, as needed
Sterile rinse water

SECOND PERIOD

Dissecting microscope

PROCEDURE First Period

1. Set up the filtration equipment. Remove wrappers as each piece is fitted into place. Why shouldn't all the wrappers be removed at once? _____
   a. Attach the filter trap to the vacuum source. What is the purpose of the filter trap? _____
   b. Place the filter holder base (with stopper) on the filtering flask. Attach the flask to the filter trap.
      Disinfect the forceps by dipping in alcohol and burning off the alcohol. Keep the beaker of alcohol away from the flame.
   c. Using the sterile forceps, place a filter on the filter holder. Why must the filter be centered exactly on the filter holder?
   d. Set the funnel on the filter holder, and fasten it in place. Filter the sample.

2. Filter the sample.
   a. Shake the water sample well to resuspend all material, and pour or pipette a measured voume into the funnel. Your instructor will help you determine the volume.* *(For samples of 10 ml or less, pour 20 ml of sterile water into the funnel first.)*
   b. Turn on the vacuum, and allow the sample to pass into the filtering flask. Leave the vacuum on.
   c. Pour sterile rinse water into the funnel. Rotate the funnel while pouring to wash bacteria from the sides of the funnel. *(Use the same volume as the sample.)* Allow the rinse water to go through the filter. Turn the vacuum off.

3. Inoculate the filter (Figure 3).
   a. Carefully remove the filter from the filter holder using sterile forceps. Why does the filter have to be "peeled" off? _____
   b. Carefully place the filter on the Endo agar. Do not bend the filter; place one edge down first, then carefully set the remainder down. Do not leave air spaces between the filter and agar. Place the filter on the agar as it was in the filter holder.

4. Invert the plate and incubate it for 24 hours at 44.5°C.
5. Repeat steps 1, 2, and 3 using the same water source. Place the filter on Enterococcus agar. Invert the plate, and incubate it for 48 hours at 35°C.

PROCEDURE Second Period

1. Examine the Endo plate using a dissecting microscope. On Endo agar, conforms will form red colonies with a green metallic sheen. Count plates with 20 to 80 coliform colonies, and not more than 200 colonies of all types.
2. Examine the *Enterococcus* plate using a dissecting microscope. On Enterococcus agar,

**Suggested sample volumes: lakes and wells, 50-100 ml; treated sewage, 0.1-10 ml; rivers and stormwater runoff, 0.1-10 ml.*

enterococci will form red colonies. Count plates with 20 to 60 *Enterococcus* colonies, and not more than 200 colonies of all types.

3. Calculate the bacteria in the original water sample:

Number of fecal coliforms (or enterococci) per 100 ml of water =

$$100 \times \frac{\text{Number of coliform (or enterococci) colonies}}{\text{Volume of water filtered}}$$

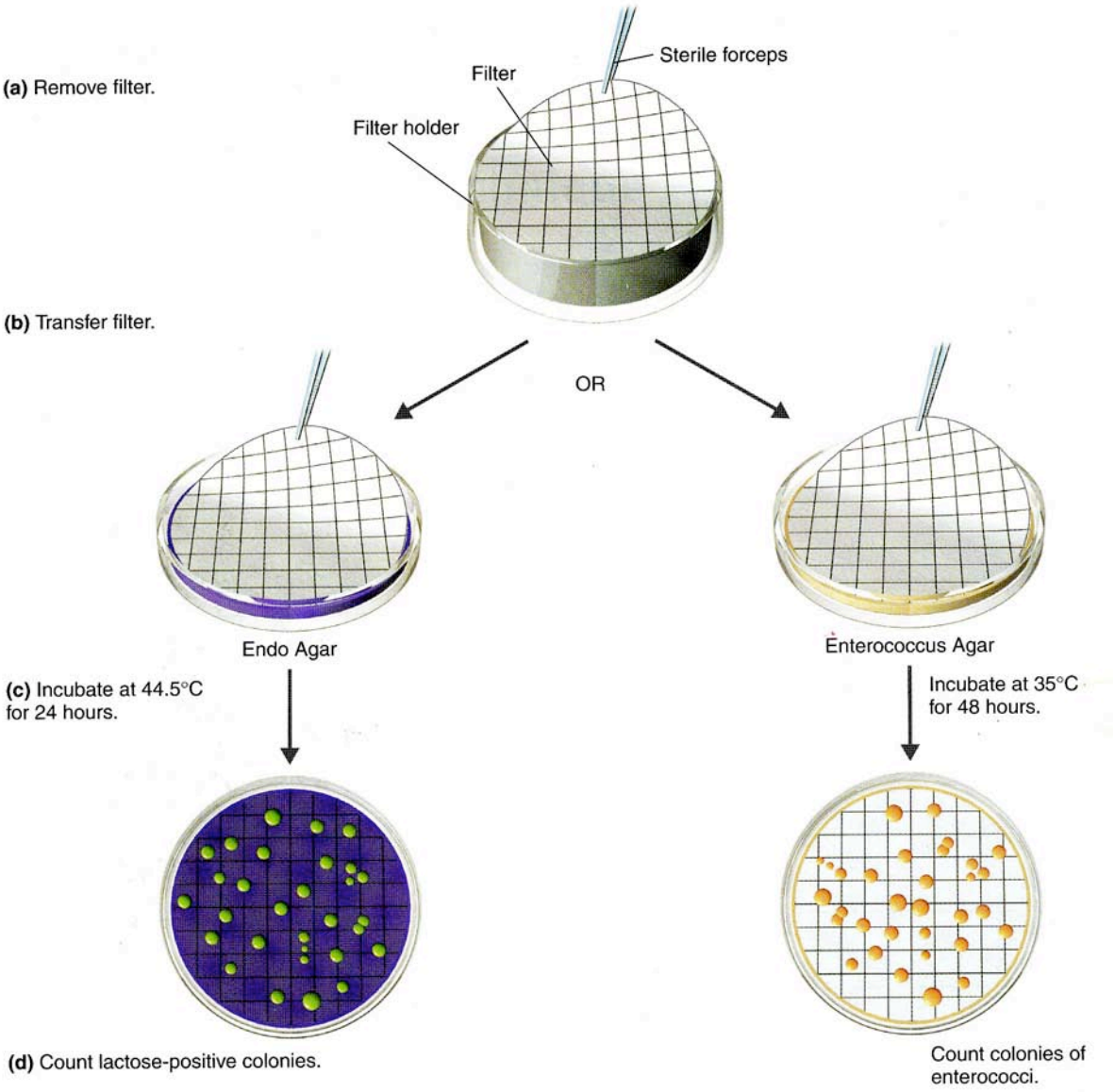

Figure 3. Inoculation. Using sterile forceps, remove the filter from the filter holder. Place the filter on the culture medium, gradually laying it down from one edge to the other.

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Name _____

Date _____

Purpose_____

_____

_____

Results

Water sample source: _____

Fecal Coliforms	Enterococci
Amount of water tested_____	Amount of water tested_____
General appearance of colonies on Endo agar	General appearance of colonies on Entero-cocus agar
Number of fecal coliform colonies_____	Number of *Enterococcus* colonies _____
Number of fecal coliforms/100 ml (MPN	Number of enterococci/100 ml (MPN

Data from water samples tested by other students:

Sample	Fecal Coliforms/100 ml	Enterococci/100 ml

Conclusions

1. Which water sample(s) is (are) potable?_____

2. Which water sample(s) is (are) contaminated with fecal material? _____

Questions

1. What basic assumption is made in this technique if the number of bacteria is determined from the number of colonies?

2. Why is the membrane filter technique useful for a sanitarium working in the field?