

## Membrane Filtration for Counting Bacteria \*

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 (see Figure 3). Filters with a variety of pore sizes are available. Pores of 0.45  $\mu\text{m}$  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 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% NaCl, 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).



Figure 1. Endo agar

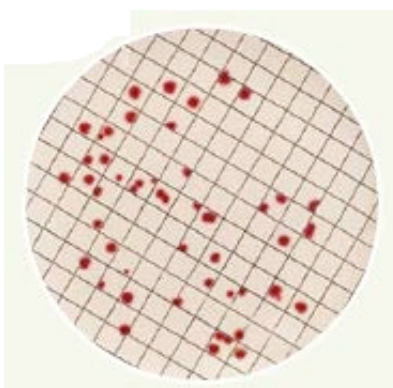


Figure 2. Enterococcus agar

Table 1. Composition of Endo agar

Tryptone	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%

Table 2. Composition of Enterococcus agar

Tryptone	2.0%
Yeast Extract	0.5%
Glucose	0.2%
Dipotassium phosphate	0.4%
Sodium azide	0.04%
Agar	1.0%
Triphenyltetrazolium chloride (TTC)	0.01%

\* T. Johnson and C. Case. *Laboratory Experiments in Microbiology*, 9<sup>th</sup> edition. San Francisco: Benjamin Cummings, 2010.

## PROCEDURE

1. Set up the filtration equipment (Figure 3a). 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 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.
2. Filter the sample.
  - a. Shake the water sample well to resuspend all material, and pour or pipette a measured volume into the funnel.\* (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 through 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 3b).
  - 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 agar. Do not bend the filter; place one edge down first, then carefully let 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.

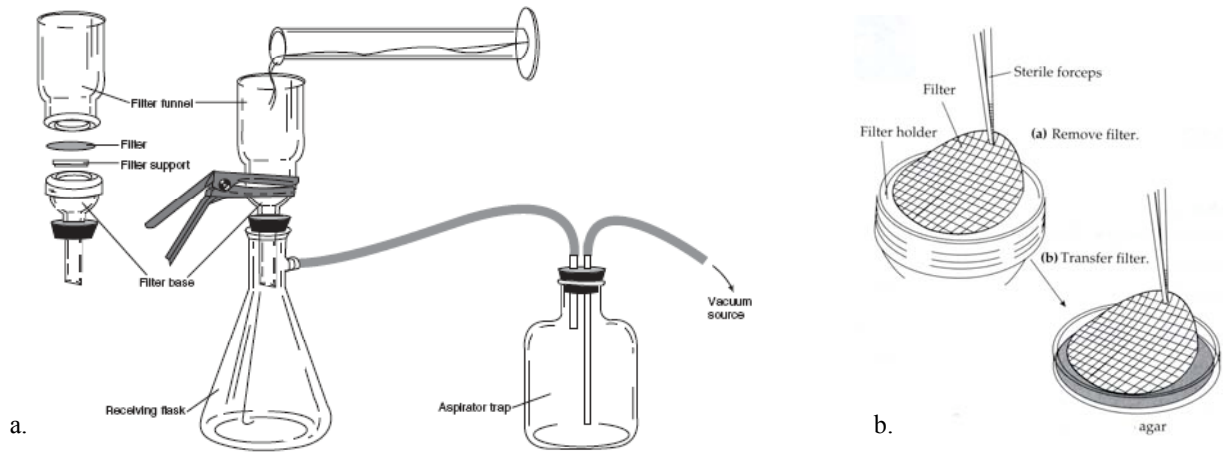


Figure 3. Membrane filtration apparatus and filter transfer

\* Suggested sample volumes: lakes and wells, 50-100 ml; treated sewage, 0.1-10 ml; rivers and storm water runoff, 0.01-1 ml.