Laboratory (4) Water Microbiology Dr Duaa Suhail

Membrane Filtration Technique

Method Definition:

It is a reliable technique for the detection of coliforms in water utilizing the membrane filter.

Method Principle:

-This method is based on the use of a highly porous cellulose- ester membrane.

-The filter disks are 150 µm thick, have pores of 0.45 µm diameter, and have 80% area perforation. Eighty percent area perforation facilitates rapid filtration.

-The pore structure enables a large volume of water or aqueous solution to pass through rapidly under pressure, but prevents the passage of any bacteria present in the sample which are larger than 0.47 µm.

-All bacteria present in the sample will ne retained directly on the filter's surface. The membrane is then placed on an absorbent pad saturated with liquid with liquid growth medium and incubated for 22 -24 hrs. The organisms on the filter disk will form colonies that can be counted under the microscope.

-If a differential medium such as *m* Endo MF broth is used, coliform will exhibit a characteristic golden metallic sheen.

Method Advantages:

1. Higher degree of reproducibility of results.

2. Greater sensitivity since larger volumes of water can be used.

3. Shorter time for getting results.

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Method Requirements:

1. Vacuum pump or water aspirators.
2. Membrane filter assemblies (Sterile).
3. Side arm flask (1000 ml) and rubber hose.
4. Sterile graduates (100 ml or 250 ml).
5. Sterile plastic Petri dishes (50 mm diameter).
6. Sterile membrane filter disks (packed with filters).
7. Sterile water.
8. Pipettes (5 ml).
9. Bottles of *m* Endo MF broth (50 ml).
10. Water samples.

Method Working Steps:

1. Prepare a small plastic Petri dish as follows:

a. With a flamed forceps, transfer a sterile absorbent pad to a sterile plastic Petri dish.
b. Using a 5 ml pipette, transfer 2 ml of *m* Endo MF broth to the absorbent pad.

2. Aseptically, assemble a membrane filtering unit as follows:

a. Insert the filter holder base into the neck of a side-arm flask.
b. With a flamed forceps, place a sterile membrane filter disk, grid side up, on the filter holder base.
c. Place the filter funnel on the top of the membrane filter disk and secure it to the base with the clamp.

3. Attach the rubber hose to a vacuum source (pump or water aspirator) and pour the appropriate amount of water into the funnel under aseptic conditions. Use a sterile graduate for measuring the water.

Note: The amount of water used will depend on water quality. No less than 50 ml should be used. Water with few bacteria and low turbidity permit samples of 200 ml or more.

4. Rinse the inner sides of the funnel with 20 ml of sterile water.

5. Disconnect the vacuum source, remove the funnel and carefully transfer the filter disk with sterile forceps to the Petri dish of *m* Endo MF broth. Keep grid side up.

6. Incubate at 35º C for 22-24 hrs. Don't invert.

7. After incubation, remove the filter from the dish and dry for 1 hour on absorbent paper.

8. Count the colonies on the disk with low-power magnification. Ignore all colonies that lack the golden metallic sheen. Record your count.

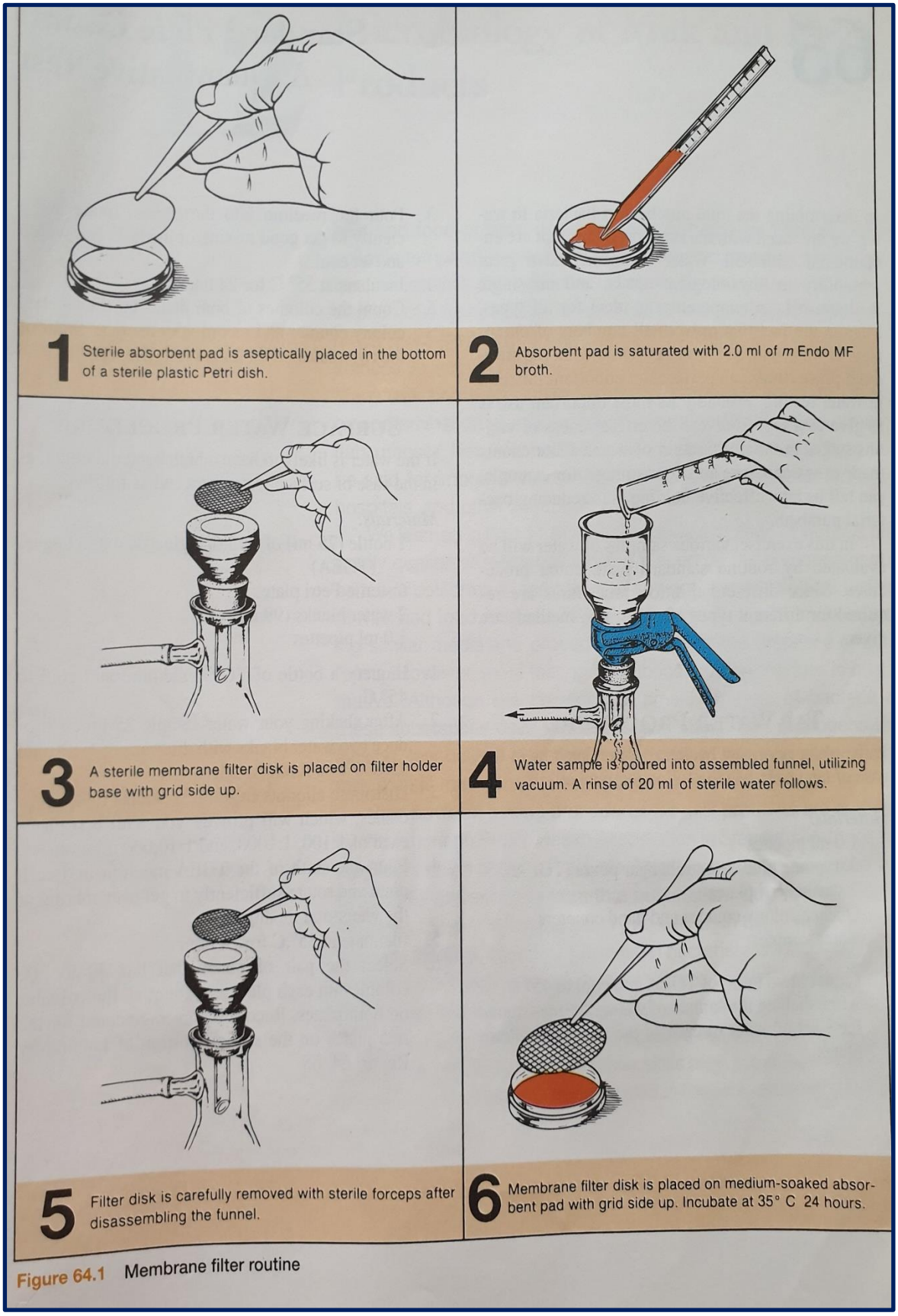

Figure 64.1 Membrane filter routine