Student's name and surname .....

Group .....

Laboratory work in microbiology.

Drinking water microbiological parameters.

The quality of drinking (potable) water is certainly an extremely important matter. A variety of agents causing gastroenteritis are carried in water supplies (see *theoretical material N.2*), including typhoid and dysentery.

The coliform group, which includes *Klebsiella*, *Escherichia*, *Citrobacter* and *Enterobacter*, are facultatively anaerobic, nonspore-forming, gram-negative rods that ferment lactose with gas formation within 48 hr at 35◦ C. One of the most common organisms that meets these criteria is *Escherichia coli* – a component of the normal microbiota of the gastrointestinal tract of warn-blooded vertebrates and, therefore, a component of feces. The coliforms serve as indicator organisms, in that they indicate the presence of sewage pollution (see *theoretical material N.3*).

This laboratory work presents main method for detection of pollution of water with microorganisms: membrane – filter method.

Membrane- filter method.

This technique utilizes membrane filters that have a pore size of 0.45 µm. When the water sample is filtered via suction through the membrane, microorganisms larger than 0.45 µm are trapped on the surface of the filter. The filter is then transferred to a media-saturated pad in small Petri dish. Nutrients can diffuse from the pad to the filter surface and may provide nutrients for the cell division or growth of any microbe trapped on the filter. An organisms trapped on the filter can eventually give rise to a colony on the surface of the filter. Depending on the type of media placed on the absorbent pad or in the plate, the enumeration of a number of different organisms is possible.

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In this laboratory work, the following counts will be determined:

1) the total heterotrophic bacterial count, using m-Heterotrophic Plate Count Agar (m-HPC) as the nutrient.

2) The total coliform count (TCC), using m-endo medium.

3) The faecal coliform count, using m-FC broth.

Procedure.

1. Attach the filter apparatus to the vacuum device (Figure 1).

2. With sterile forceps, place a membrane filter on the platform bace of the filter apparatus.

3. Pour about 20 mL of the sterile distilled water into the filter funnel.

4. Filter 100 mL of drinking water.

5. If using a diluted sample, start with the highest dilution and add to the filter funnel containing the sterile distilled water. Filter by applying a vacuum.

6. Turn off the vacuum and wash the walls of the filter funnel with at least 40 mL of sterile distilled water.

7. Turn on the vacuum to filter the wash. It is important to rinse to the filter surface any organisms that might be adhering to the walls of the filter funnel.

8. Place absorbent pads in the 60x15 mm Petri plates and label appropriately.

9. Add 2.0 mL of the proper medium to each absorbent pad. In the case of the total heterotrophic count, no pads are necesary since an agar base is used.

10. Using sterile forceps, transfer the filter to the appropriate saturated pad in the Petri dish. For the total heterotrophic count, place the filters directly onto the agar surface.

11. Incubate the heterotrophic- count plates at 28° C for two to five days. The total coliform plates should be incubated at 35° C for 22 to 24 hr.

12. After incubation, remove all filters from the plates and allow them to dry on absorbent paper for 1 hr.

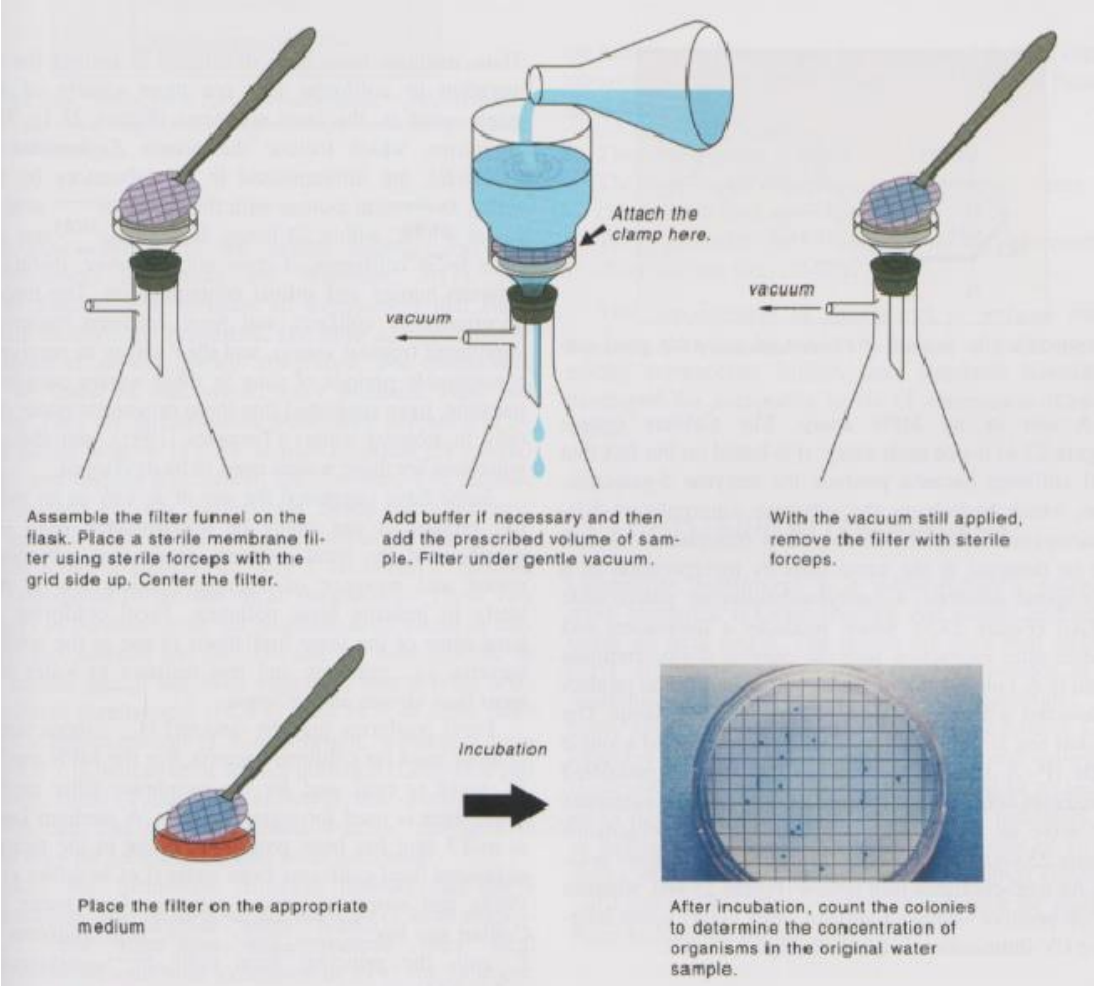

Figure 1. Membrane- filter method [3].

On the total heterotrophic-count filters count all colonies of the filter when there are 2 or fewer colonies per square. For 3 to 10 colonies per square, count 10 squares and obtain an average count per square. For 10 to 20 colonies per square, count five squares and obtain an average count per square. Multiply average count per square by 100 times the reciprocal of the dilution to give colonies per mililiter of water.

The coliforms is a group of bacteria producing colonies that are pink to dark red in colour, with a metallic sheen on total coliform filters. The sheen can present itself in form of concentration in the conter of colony, or it may cover the entire colony. It is the non-coliform organisms that produce the sheen colonies as a rule. For coliform counts of more than 5 per 100 mL of drinking water, verify a minimum of 5 colonies. To verify, the use of lauryl tryptose broth and brilliant-green lactose broth is required. A suspected coliform colony needs to be emerged into a tube of lauryl tryptose broth containing a

fermentation tube (Durham tube). Indication of a positive presumptive test for coliforms is gas or acidic growth in the tube within 48 hr. Transfer a loopful of culture (using a sterile loop) to a tube with brilliant-green lactose bile broth. The test for coliforms can be confirmed as positive if gas is formed in the inverted Durham tube within 48 hr.

Colonies produced by fecal coliform bacteria are various shades of blue. Non-fecal coliforms are grey to cream colored. Normally, few non-fecal coliform colonies will be observed on the fecal coliform medium because of the selective action of the elevated temperature and the presence of rosolic acid. Record the densities as fecal coliforms per 100 mL of water.

Review Questions.

1. Were any of the water samples polluted with human waste? How was this determined?

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2. What are some of the criteria for indicator bacteria?

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3. Define, what are coliforms?

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4. What are the main differences between m-Heterotrophic Plate Count Agar and m-endo Agar medium?

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