

Isolation and Enumeration of water Microorganisms

Water receives microorganisms from air, soil, sewage, organic wastes, dead plants and animals...etc.

Microbial enumeration tests for water samples are essential for assessing water quality and ensuring safety for consumption and use. These tests involve measuring the number of microorganisms, such as bacteria, fungi, and protozoa, present in a water sample. Common methods include the plate count technique, membrane filtration, and most probable number (MPN) method.

Types of media used for water samples:

General media: Nutrient agar

For coliforms bacteria : MacConkey agar

For fungi and yeasts: Sabouraud Agar

Procedures:

- **Collect the samples:** Collect water samples from various sources (e.g., river, sea, sewage, drinking water) in sterile containers.
- Make serial dilution (10^{-x}) of water samples according to the source as the follows:

Sewage water **6** dilutions

River water **5** dilutions

Tank water **3** dilutions

Drinking water **1** dilution

- Suspend 1 ml of water sample in 9 ml D.W. to get 10^{-1} , then transfer 1 ml of first dilution to another tube to prepare the second dilution 10^{-2} , the other tubes remaining dilutions are prepared in the same way (10^{-3} to 10^{-6}).
- Then transfer 1 ml from the selected dilution to sterile Petri dishes, after that pour the melted agar into the dishes and thoroughly mix with the sample by a gentle rotation of the dish.
- Incubate the Petri dishes for 24 -48 hr at 28-37°C.

Methods in bacterial counting:

Direct methods

1-Direct Microscopic Count : is a count of cells including dead and live cells by slide or chamber .

2-Standard plate count (viable count): is a count of only those cells that are alive in the sample. The standard plate count method consists of diluting a sample with diluents . After Serial dilution (a set of dilutions) we obtain a culture plate with a countable number of separate colonies.

Choosing plates that have colony between 30 and 300 colonies.

- ❖ The number of bacteria in 1 ml of the original sample can be calculated as follows:
- ❖ Total No. of Bacteria (**CFU**)* /ml= Average No. of colonies x Reciprocal of Dilution.

Suppose that you count 150 colonies in 10^{-5} dilution.

$$\text{CFU/ml} = 150 \times 10^{-5}$$

$$= 150 \times 10^5 \Rightarrow \text{So CFU/1ml} = 150 \times 10^5.$$

***CFU means** = colony-forming unit, a term estimate the number of viable microbial cells—such as bacteria or fungi—in a sample that are capable of multiplying to form a visible colony.

3-Most probable number (MPN).

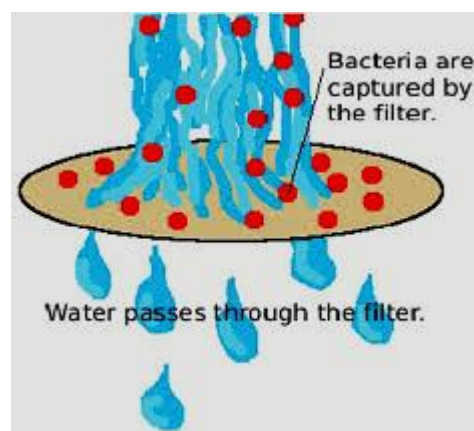
4- Membrane filter technique:

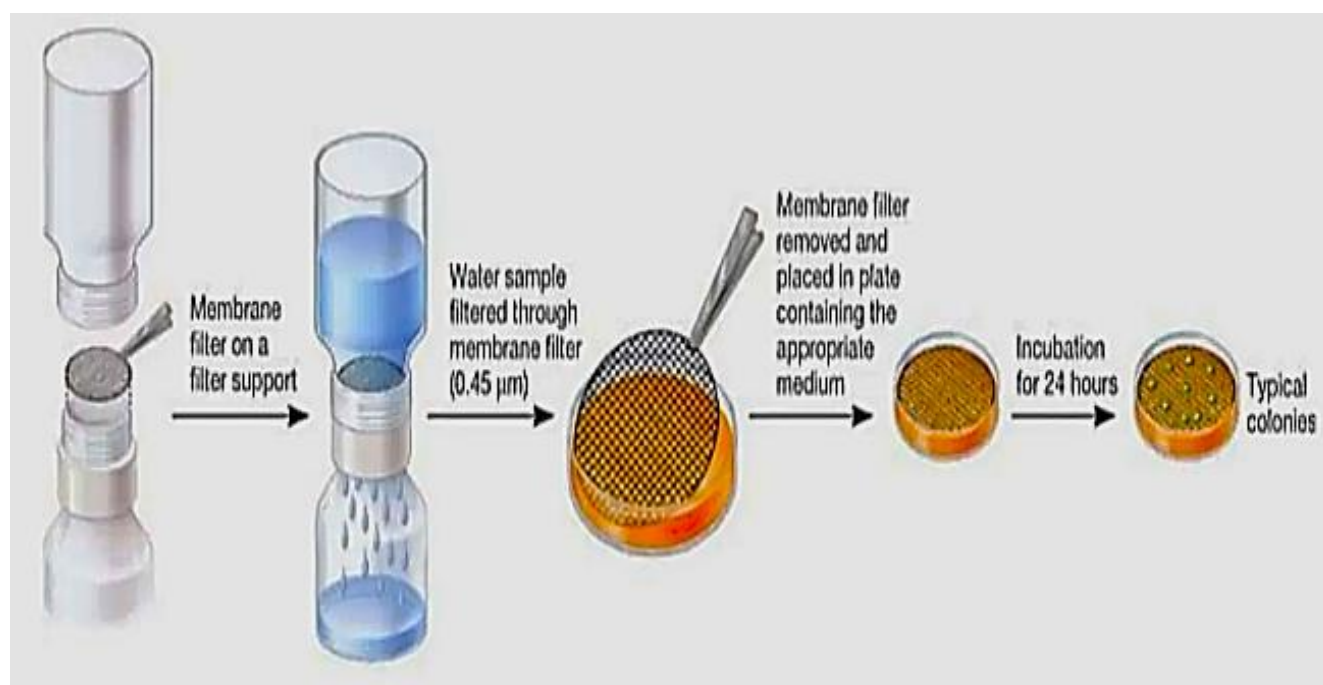
Process: A specific volume of water is passed through a sterile membrane filter that traps the microorganisms. The pore size of whatman is small $0.45 \mu\text{m}^*$ enough to capture bacteria.

* μm = Microns, also known as micrometers (represented as μm) are a length of measurement equal to one millionth of a meter.

Incubation: The filter is placed on top of a nutrient-rich culture medium in a petri dish.

Enumeration: After incubation, the colonies that grow on the filter are counted to determine the microbial concentration.





Remember :

Microliter to Milliliter Conversion Table

Microliter [μL]	Milliliter [mL]
10 μL	0.01 mL
100 μL	0.1 mL
1000 μL	1 mL