Isolation and Enumeration of Water Bacteria

Bacteria existing in water could be divided into several groups:

1. Bacteria naturally present in water, these are mostly Gram-negative bacteria include species belonging to the following genera: *Acinetobacter, Cytophaga, Flavobacterium, Pseudomonas,* and *Chromobacterium*. Gram-positive bacteria might be also available such as *Micrococcus, Bacillus*.

2. Bacteria from soil mostly the genus *Bacillus*.

3. Bacteria from human and animal gut as a result of water pollution with human and animal waste such as coliform bacteria (*Escherichia coli*).

4. Bacteria can also enter water through the air or with rainwater such as *Streptomyces, Bacillus*.

Total Bacterial Count Techniques of Water Samples

There are various techniques for the enumeration of bacteria in a given sample. Viable cells count allows to detect the number of actively growing and dividing cells in a sample.

A- Plate Count Technique

Method Definition: The plate count method is one of the most commonly used procedure as it allows the enumeration of viable cells. This method depends on bacteria forming distinct colonies on a culture medium which can be easily counted.

Method Principle: The principle of this technique is that "when a sample containing bacteria is cultured, every viable bacterium develops into a visible colony on an agar medium". The number of colonies, thus, is same as the number of the organisms present in the sample.

To ensure that an appropriate number of colonies will be generated, the original sample must be diluted and several serial dilutions are normally performed and cultured. Colonies grown from each dilution can be counted between the range of 30-300 colonies. Less than 30 colonies make the interpretation statistically unreliable and more than 300 colonies often results in overlapping colonies and errors in counting (imprecision).

Method Requirements

- Water Sample
- 9 ml dilution blanks in distilled water (DW)
- Sterile 1 ml (1000 µl) Micropipettes
- Sterile Petri plates
- Agar medium
- Colony counter

Method Working Steps

1. Collect water samples from various sources such as river water, tap water…etc.

2. Label the dilution blanks (each contain 9 ml DW) as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.

3. Prepare the initial dilution by adding 1 ml of the water sample into 9 ml dilution blank labelled 10^{-1} thus diluting the original sample 10 times (1/10 and is written as 10^{-1} or 1;10).

4. From the first dilution, transfer 1 ml of suspension to the dilution blank 10^{-2} with a micropipette diluting the original specimen to 100 times (1/100 or 10^{-2}). Repeat this procedure for the next blank dilutions 10^{-3}, 10^{-4} and 10^{-5} diluting the original sample to 1000 (1/1000), 10000 (1/10000) and 100000 (1/100000) times, respectively.

Note: Serial dilutions are series of sequential dilutions used to reduce a dense culture of cells to a more appropriate concentration. Each dilution will reduce the concentration of bacteria by a specific amount.

5. Transfer 1ml from each dilution to a sterile Petri plate (in triplicate for each dilution).

6. Pour a molten and cooled (45-50 ºC) suitable agar medium in each Petri plate containing the transferred volume of diluted sample. Mix the content of each plate by rotating gently to distribute the cells throughout the medium, and allow the plates to solidify.

Note: The pour plate method is used when the analysis is looking for bacterial species that grow poorly in air, for example water samples.

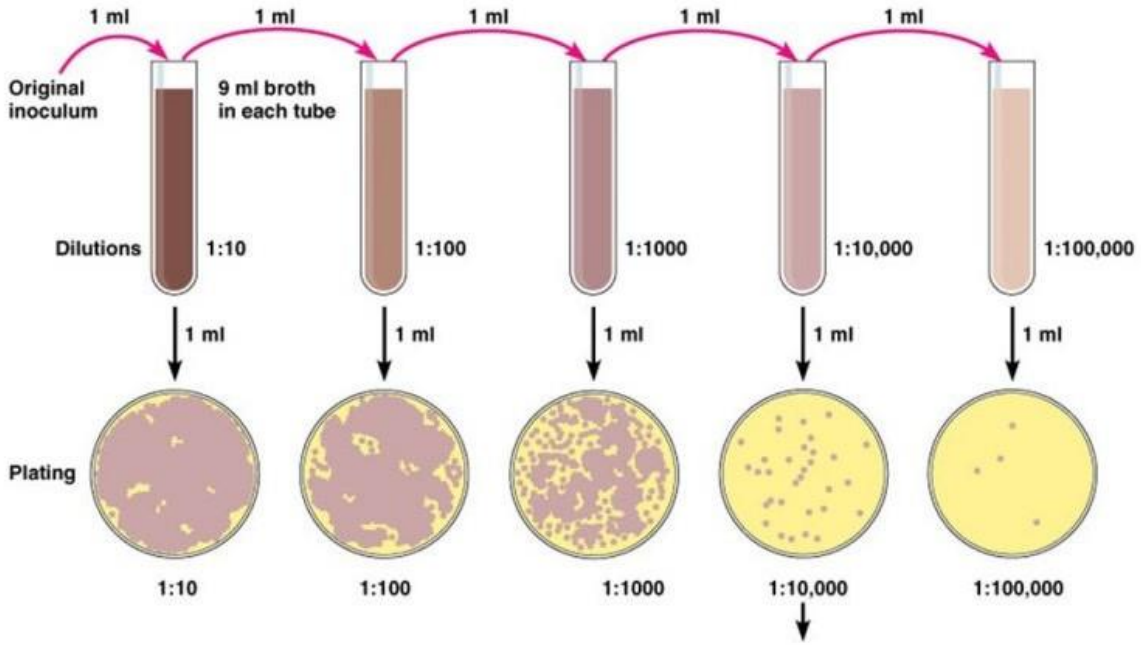
7. Incubate the first set of plates at 25 ºC for 7-14 days, incubate the second group of plates at 37 ºC for 24-48 hrs (two days).

8. After incubation, count the colonies grown in plates within the range of 30-300 colonies.

9. Calculate the number of bacterial cells per 1ml of the original water sample following this equation:

Number of cells in 1ml of the sample = Number of counted colonies (average of three replicates) X dilution factor (inverted dilution)

The total number of colonies is referred to as the Total Viable Count (TVC). The unit of measurement is cfu/ml (or colony forming units per milliliter).



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)