Enumeration of Bacteria
Standard Plate Count (Viable)

A standard plate count is a method used to determine the number of viable bacterial cells per unit volume of a sample by plating a very dilute portion of that sample and counting the number of colonies it produces. The colonies are referred to as **colony forming units (CFU)**.
The inoculum that is transferred to the plate contains a known proportion of the original sample because it is the product of a serial dilution.

A serial dilution is any dilution where the concentration decreases by the same quantity in each successive step (tubes containing a known volume of sterile diluent-water, saline, or buffer).
The series begins with a sample containing an unknown concentration of cells and ends with a very dilute mixture containing only a few cells.
Each dilution blank in the series receives a known volume from the mixture in the previous tube and delivers a known volume to the next, typically reducing the cell density by 1/10 or 1/100 at each step.
For example, if the original sample contains 1,000,000 cells/mL, following the first transfer, the 1/10 dilution (1ml into 9ml) in dilution tube 1 would contain 100,000 cells/ml. In the second dilution tube2, the 1/100 dilution would reduce it further to 10000 cells/ml. Dilutions are expressed in scientific notation, a 1/10 is written as 10^{-1} and a 1/100 is written as 10^{-2}.A known volume of appropriate dilutions is then spread onto agar plates to produce at least one countable plate.

A countable plate is one that contains between **30** and **300** colonies. A count lower than 30 colonies are considered statistically unreliable and greater than 300 is typically too many to be viewed as individual colonies.
A colony counter with a magnifying lens is useful if the colonies are small.

Standard Plate Count includes:
Spread plate method (surface viable count)
Pour plate method

Advantages of standard plate count
1. Plate counts assume that every colony is founded by a single cell.
2. That cell must have been alive in order to grow and form a colony.
3. In viable plate method only bacteria can be counted.

Disadvantages of plate counts
1. They require lengthy incubation for colonies to become visible.
2. Cell clumping (growth together) can lead to an undercount of viable cells.
3. Prevention of crowding often requires serial dilution (Depending on your organism, and other circumstances, you will want to have no more than 300 to 500 colonies per plate (e.g., for big colonies 300 will be many, for small colonies 500 may still work).
4. Too few cells requires concentrating, e.g., by centrifugation or filtration.

Procedure

1. Prepare the bacterial suspension from your sample (if the sample liquid you take a few amount (ml) and add to a certain volume of distilled water. But if the sample is solid, you must put a certain weight of your sample and dissolve in the certain volume of distilled water).
2. Prepare the serial dilution (number of tubes; for instance 6 tubes, and labeled all tubes).
3. Add 9 ml nutrient broth or distilled water into all tube.
4. Add 1 ml from your bacterial suspension into first tube and **shake well**.
5. From first tube add 1 ml into second tube and **shake well**.
6. So from second tube to third tube as clarify in the figure (1). Therefore, discard 1 ml from the final tube.
7. Prepare nutrient agar plates for each tube.
8. Add **0.1** or 1.0 ml from each tube to select plate and spread al shown in figure (2).
9. Incubate all plates (labeled carefully).
10. Calculate the bacterial cells in 0.1 ml of tubes by the following equation:
    Bacterial cells/ 0.1 ml = No. of bacterial colonies in each plate × Inverted dilution
11. Then done this equation for all plates.
12. Finally, convert the bacterial cells from 0.1 ml to 1 ml and your sample volume.

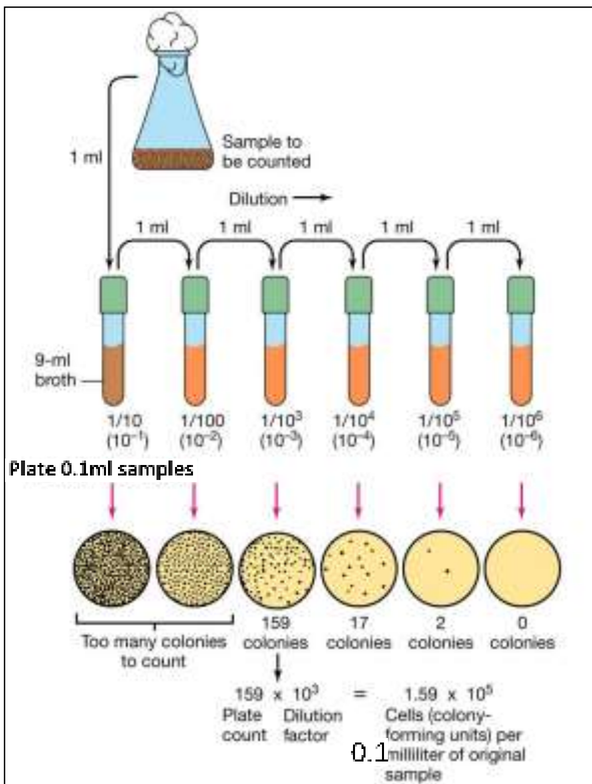

Figure (1): Procedure for viable counting using serial dilutions of the sample.

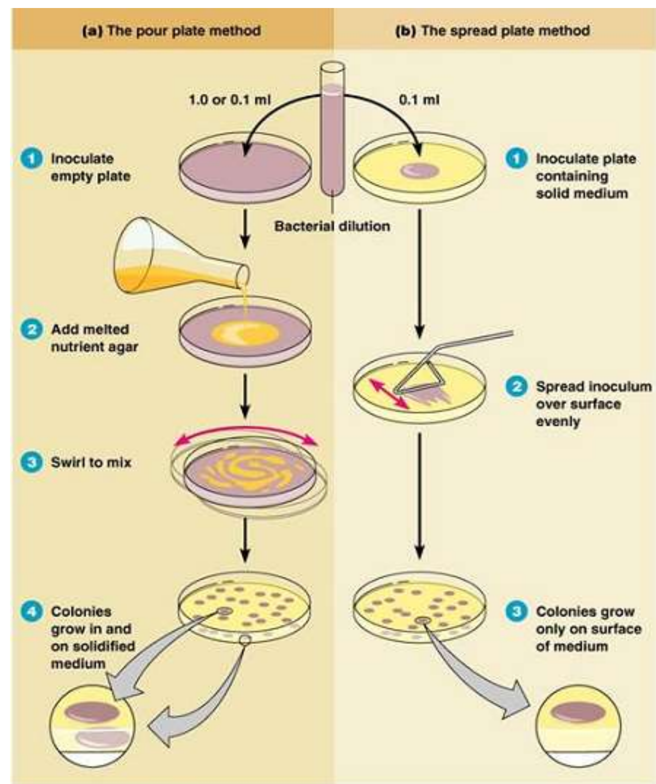

Figure (2): Two methods of performing a viable count (plate count).

For example, if plate no. 3 contained 40 colonies we can determine the concentration in the sample.

a. Bacterial cell/0.1ml = 40 CFU x10^3= 4x10^4 CFU/0.1ml

b. Bacterial cell/ml = 4x10^4 /0.1 = 4x10^5 CFU/ml

Estimation of microbial numbers by CFU will, in most cases, undercount the number of living cells present in a sample for these reasons. This is because the counting of CFU assumes that every colony is separate and founded by a single viable microbial cell. Many bacteria grow in chains (e.g. Streptococcus) or clumps (e.g. Staphylococcus).