Laboratory cultivation of microorganisms:

The Standard Plate Count (SPC): refers to a method of determining the concentration of bacteria also referred to as the aerobic plate count or the total viable count, is one of the most common tests applied to indicate the microbiological quality of food.

The Standard Plate Counts test evaluates both total aerobic bacterial and total mold and yeasts. Plate counts can be performed by membrane filtration, pour plates, or spread plate methods.

1.Pour plate method:

The pour plate technique can be used to determine the number of microbes/mL or microbes/gram in a specimen. It has the advantage of not requiring previously prepared plates, and is often used to assay bacterial contamination of foodstuffs.

The principle steps are to (procedure)

1-prepare/dilute the sample

2-place an aliquot of the diluted sample in an empty sterile plate

3-pour in 15 mL of melted agar which has been cooled to 45º C, swirl to mix well

4-let cool to solidify on a flat table top

5-invert and incubate to develop colonies

6-Each colony represents a "colony forming unit" (CFU). For optimum accuracy of a count, the preferred range for total CFU/plate is30 to 300 colonies/plate.

A colony-forming unit (CFU): is a unit used to estimate the number of viable bacteria or fungal cells in a sample. Viable is defined as the ability to multiply via binary fission under the controlled conditions.

$$\frac{\text{(Average count)}}{\text{(Dilution plated) (mL plated)}} = \text{CFU/mL}$$

Calculation: number of colonies on plate x reciprocal of dilution of sample=number of bacteria/ml (for example , if 40 colonies are on aplate of 1/10,000 dilution .then the count is 40 x 10,000=400,000/ml in sample)

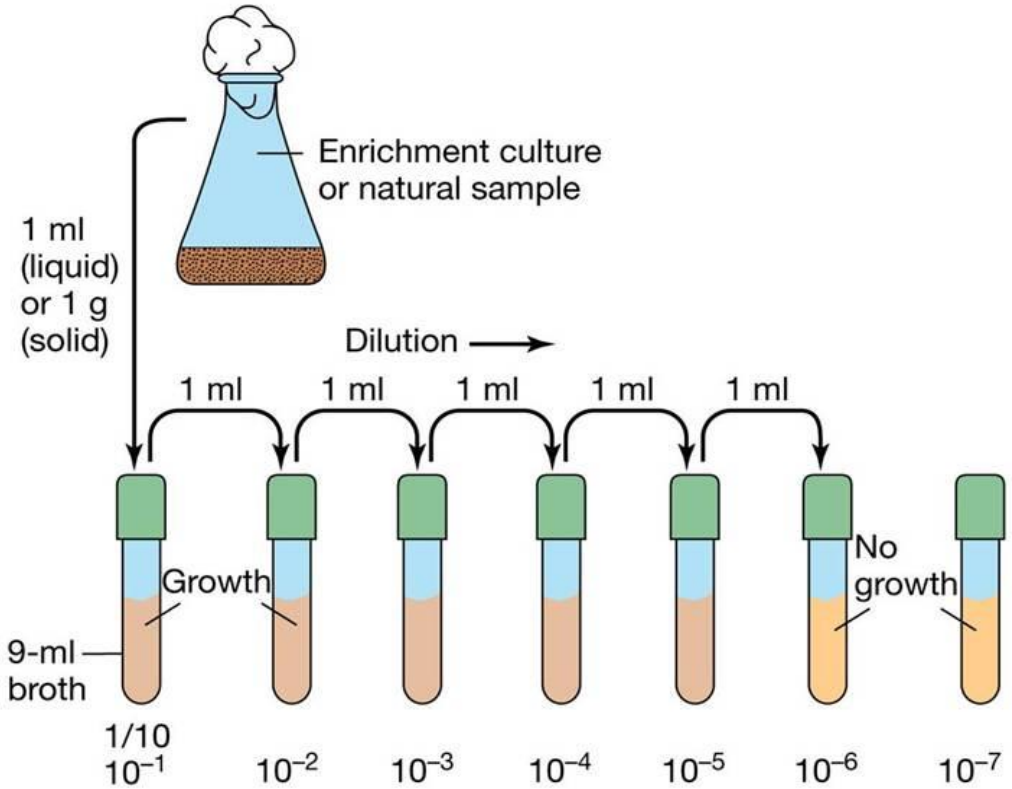
Disadvantages of Pour plate method:

1.Preparation for pour plate method is time consuming compared with streak plate/and or spread plate technique.

2.Loss of viability of heat-sensitive organisms coming into contact with hot agar.

3.Embedded colonies are much smaller than those which happen to be on the surface. Thus, one must be careful to score these so that none are overlooked.

4.Reduced growth rate of obligate aerobes in the depth of the agar



2.Spread plate: also known as lawn plates, should result in a culture spread evenly over the surface of the growth medium. The spread plate can be used for quantitative work (colony counts) if the inoculum is a measured volume, usually 0.1 cm3, of each of a dilution series, delivered by pipette.

Procedure of Spread Plate Technique:

1.Make a dilution series from a sample.

2.Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.

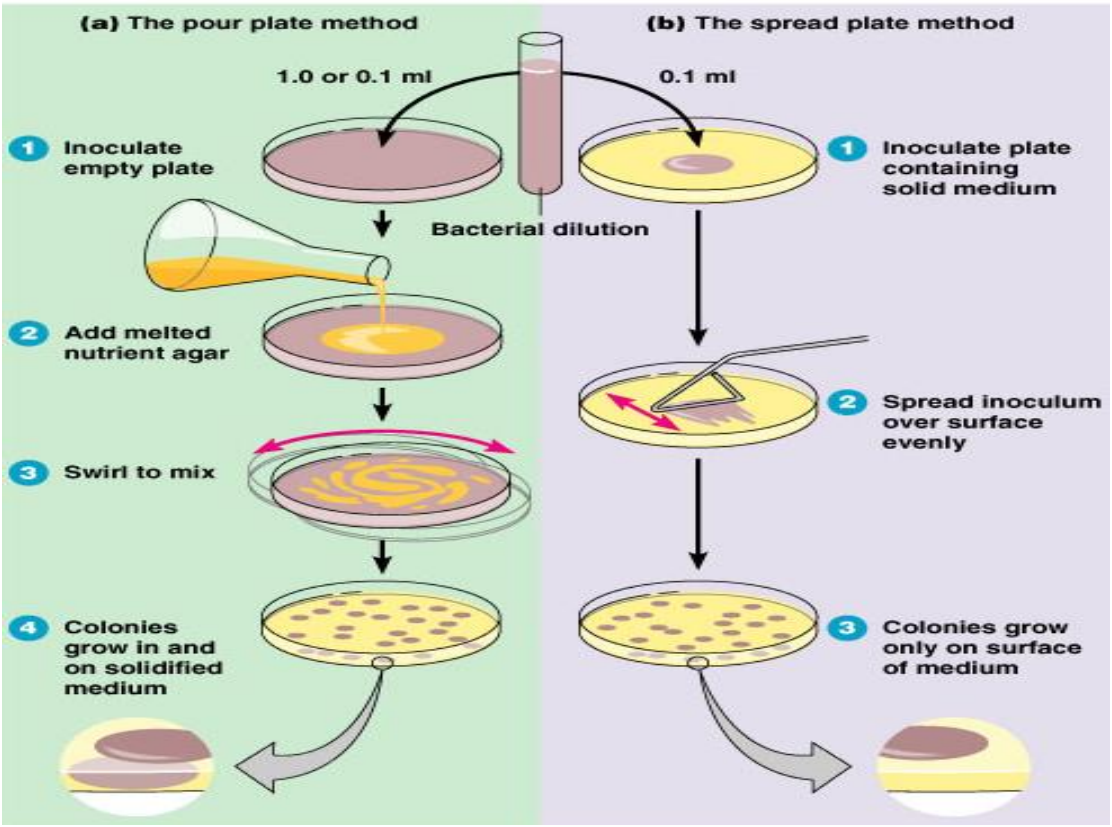
3.Dip the L-shaped glass spreader into alcohol.

4.Flame the glass spreader (hockey stick) over a Bunsen burner.

5.Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating thePetridish underneath at the same time.

6.Incubate the plate at 37°C for 24 hours.

7.Calculate the CFU value of the sample. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/mL in the original sample.


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
Bacterial dilution
1 Inoculate plate containing solid medium
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium