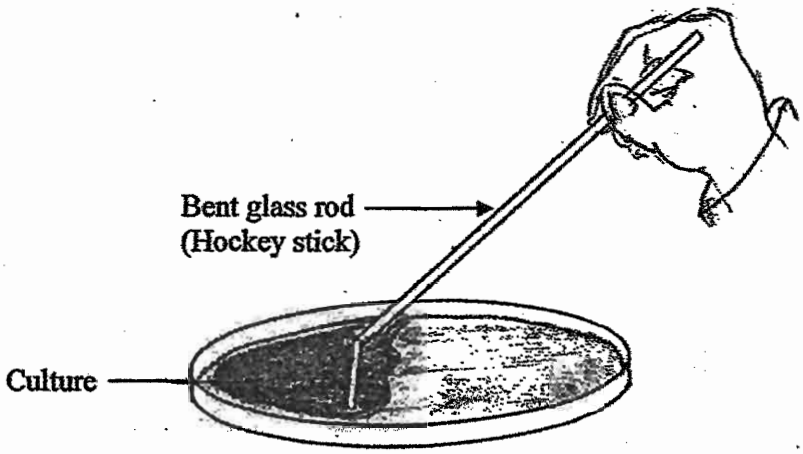
Spread Method

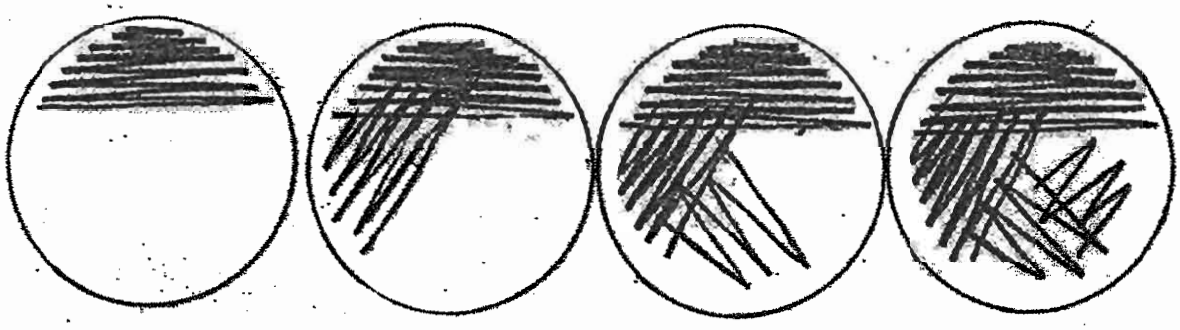
About 1 ml of a culture broth is dropped on a petri dish of solid agar medium. Using a bent glass rod, also known as a 'hockey stick', the culture is spread evenly to cover the entire plate. The plate is incubated at 37 ° C for 18 – 24 hrs. Growth of individual cultures are observed.



Sketches by Yoga Sundram

Streak Plate Method

This method is specifically used to isolate pure colonies from a mixed or contaminated culture. The purpose is to dilute or thin out the original culture so that at the end individual colonies of a pure culture is seen. Using a loop the culture is streaked on one end of a solid agar medium in a petri dish. By touching this area and streaking on another location at a different angle and repeating this two more times, the dilution is achieved. Individual colonies are seen at the end of incubation at 37 ° C for 18 – 24 hrs.

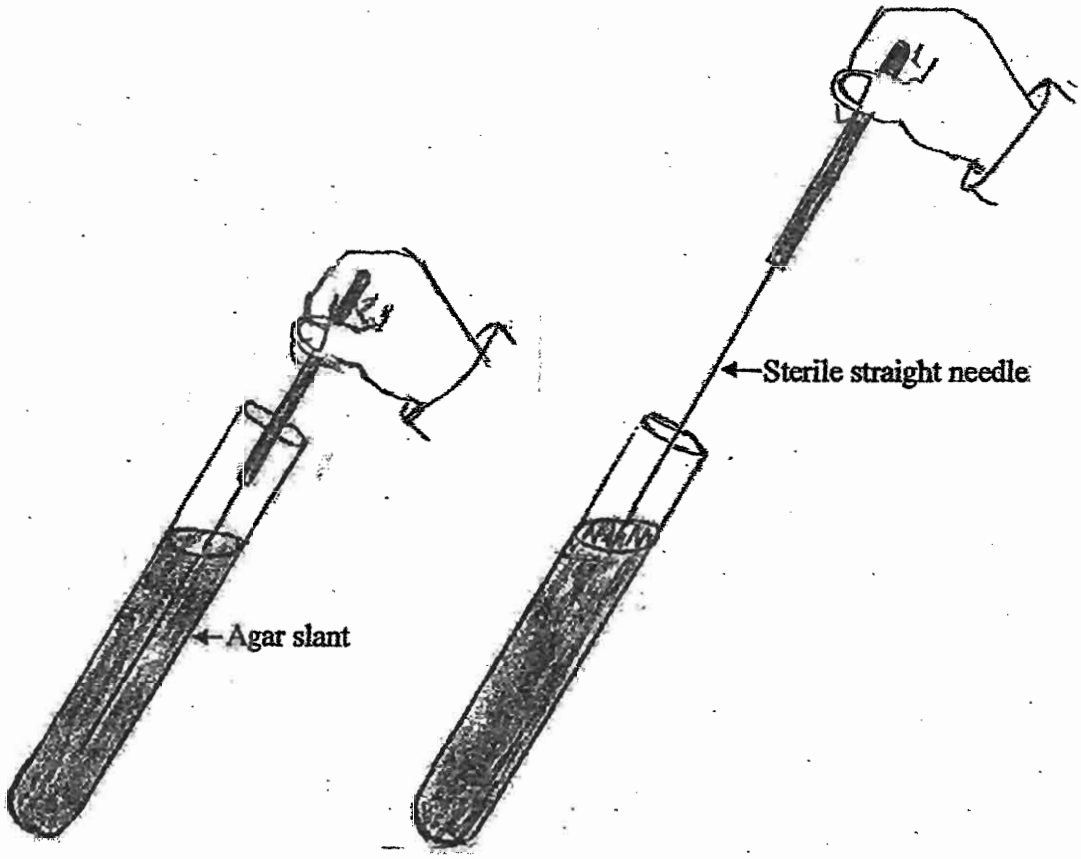


1st streak 2nd streak 3rd streak 4th streak

Sketches by Yoga Sundram

Stab and Streak Method

This method is used for identifying an unknown bacterial organism. The medium used is a differential slant agar. The culture is picked on a sterile straight needle. The agar is stabbed down the center of the tube more than halfway. The needle is withdrawn and the surface of the slant is streaked. The slant is incubated at 37 ° C for 18 – 24 hrs. Growth and color changes are observed.



Stab method **Streak method**

Sketches by Yoga Sundram

Broth Method

Bacteria may also be grown in a broth. Bacteria grow well in nutrient and other differential broths. This method is used as a growth enhancement technique prior to using differential and selective media. Some broths, such as urea broth, are used as a differential media themselves.

Serial Dilution Method

To quantify the bacterial population in a culture, the serial dilution technique is used. The original sample solution is diluted in a sequential ten fold dilutions with sterile saline solution. A known aliquot of each dilution is dropped on separate media plates. Colonies are counted on at least two dilution levels where individual colonies are observed. These numbers are used to derive at the initial concentration. This enumeration technique is widely used in research laboratories.

Enumeration of bacteria in sample.

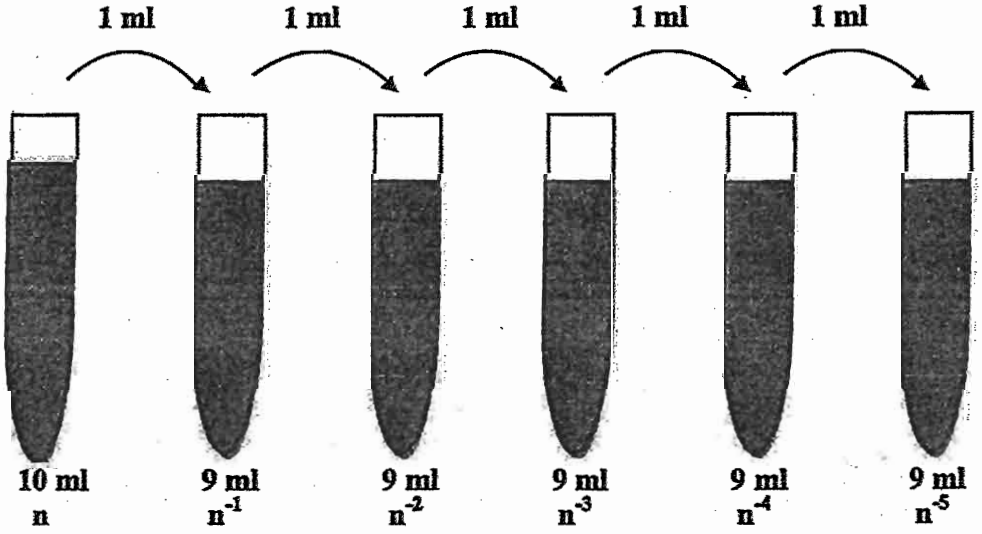
Colonies are referred to as colony forming units or CFU
Consider 5 serial dilutions are made (Ten fold sequential dilutions).

Let n be the CFU / ml of the original sample.
First dilution will have $n/10 = n \times 10^{-1}$ CFU / ml of sample
Second dilution will have $n/10 = n \times 10^{-2}$ CFU / ml of sample
Third dilution will have $n/10 = n \times 10^{-3}$ CFU / ml of sample
Fourth dilution will have $n/10 = n \times 10^{-4}$ CFU / ml of sample
Fifth dilution will have $n/10 = n \times 10^{-5}$ CFU / ml of sample and so on

Colonies are counted on two sequential dilutions where individual colonies can be seen. A colony counter may be used for this purpose.

Example: If at the fifth dilution the number of colony forming units counted on the plate was 25. Extrapolating this number:

$$n \times 10^{-5} = 25$$
$$n = \frac{25}{10^{-5}}$$
$$= 25 \times 10^{5}$$
$$\text{Therefore } n = 2500000$$
$$= 2.5 \text{ million CFU}$$
$$\text{This is represented as } = 2.5 \times 10^{6} \text{ CFU/ml of original sample}$$



Sketches by Yoga Sundram