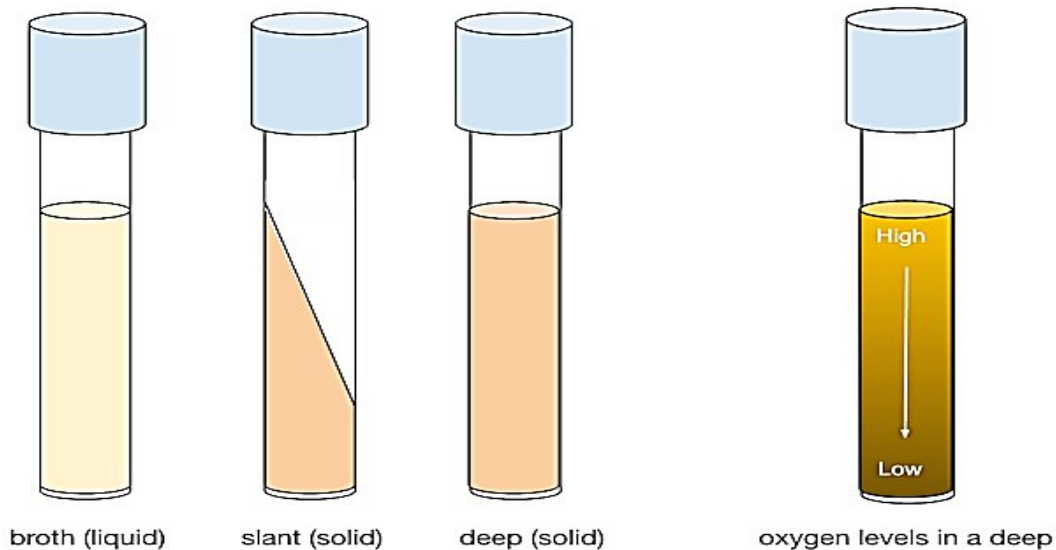


Methods of Culturing Microorganisms



Growth media can be either liquid or solid or semi-solid.

- 1- **Broth** : For growing large quantities of bacteria.
- 2- **Agar** : To obtain obvious colonies of bacteria, Agar plates are made by pouring melted agar into a petri dish. The forms of agar are : Agar plates, Agar slants and Agar deeps.
- 3- **Semi-solid media** : for detecting motile bacteria.

Methods of culturing in a solid medium culture :

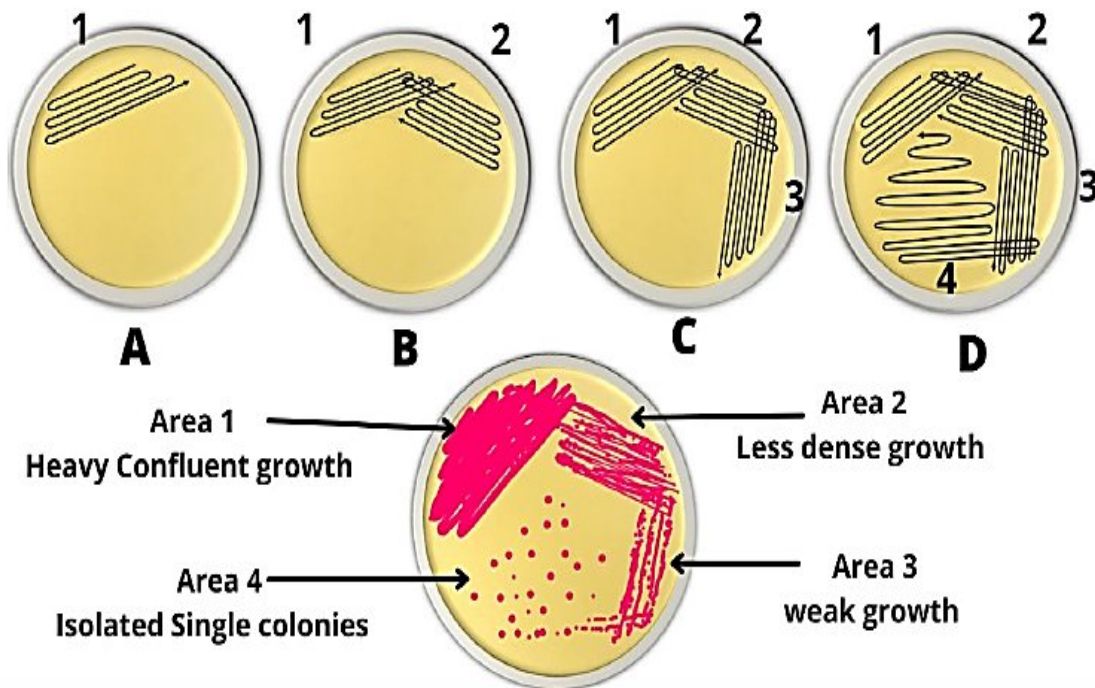
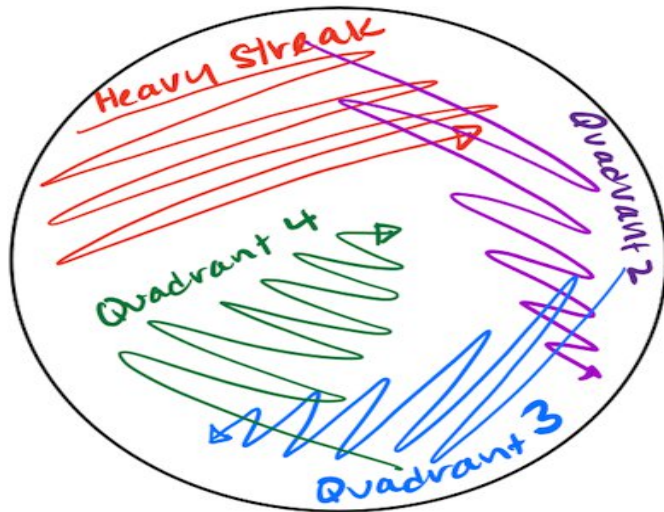
1- Streak plate method : on the surface of the plate, distribution a sample (Whether from a liquid or agar)with sterile loop or swab.

Types of Streak Plate Method

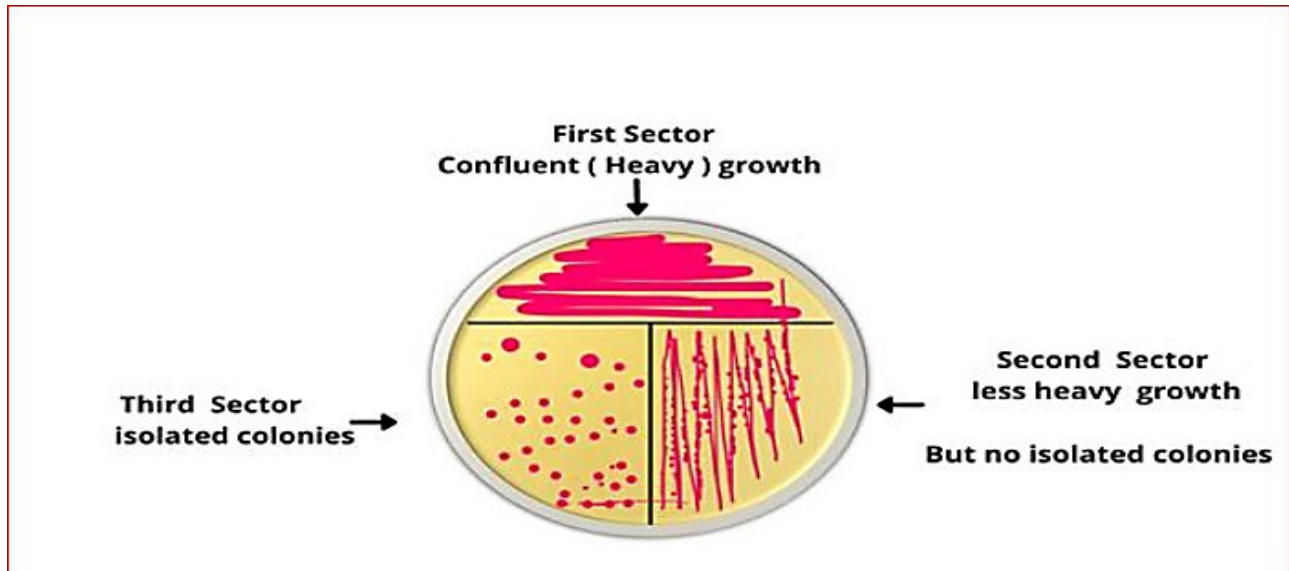
The streak plate method can be classified into 5 types: Quadrant Streaking, T-Streaking, Continuous Streaking, zigzag Streaking and Lawn or carpet method.

Quadrant Streaking: used to isolate pure colonies, to get single bacterial colonies from a mix of types of bacteria in a sample by making four areas, serially diluted streaking zones on an agar plate .

By sterilizing the inoculation loop between each quadrant, the bacterial density decreases.

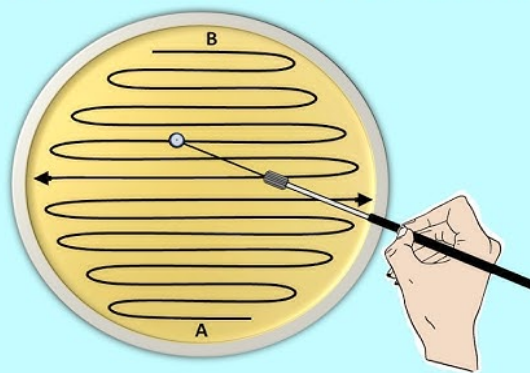


T-streaking: is a technique that divides the plate into three sections, used to isolate pure bacterial colonies from a mixed culture by diluting the inoculum on an agar plate (T-shape).

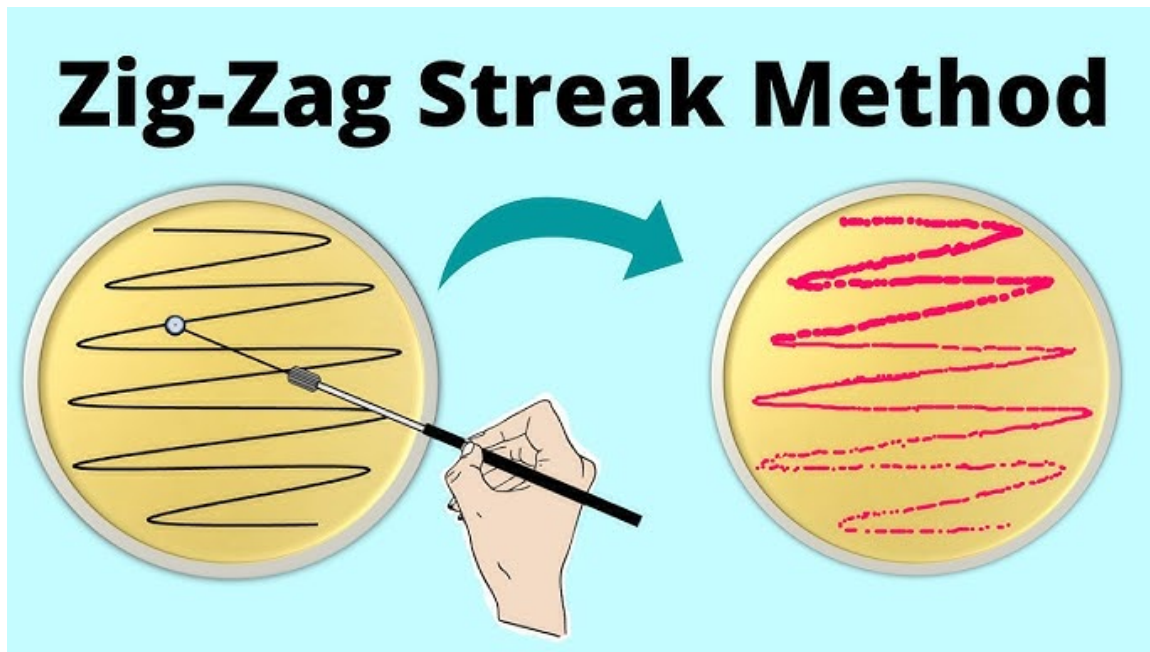


Continuous streaking: is a rapid microbiology technique used to multiply/proliferate (To increase in number) bacteria by spreading an inoculum لقاح on an agar plate in one continuous motion. Unlike quadrant or T streaking, it does not require sterilizing the loop between steps because we need to get more growth, and It is possible to use the swab.

Continuous Streak Method



Zigzag Streaking: Its purpose is the same as continuous streaking, but unlike continuous lines, the edges of the zigzag lines are sharp.



Lawn Culture or Carpet culture:

is a technique ensures the bacteria cover the entire agar surface.

Procedure: A liquid bacterial suspension is used to flood the agar plate surface.

A sterile swab is dipped into a liquid broth culture and applied to the agar surface, usually in multiple directions (like cross shaped).

Purpose / Uses :

It is useful for antibiotic sensitivity testing by disk diffusion method.

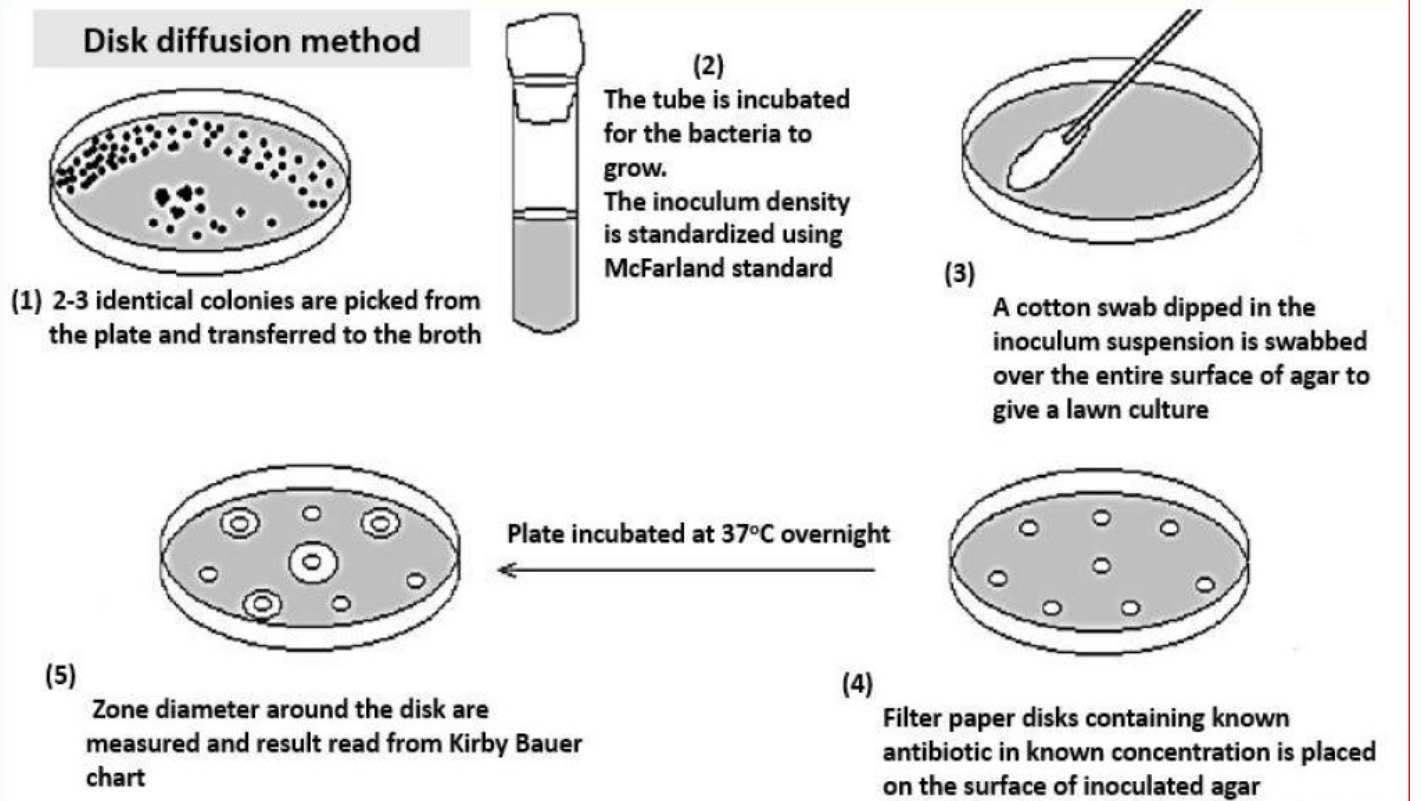
The Materials of a technique :

- Petri dishes with Mueller-Hinton agar.
- Bacterial culture.
- Sterile Swabs.
- forceps.
- Antibiotic discs 1 of each kind.

@ Why do we use Mueller-Hinton agar in antibiotic sensitivity testing?

MHA has low inhibitor content and high starch content, which absorbs bacterial toxins and allows good diffusion of antimicrobial agents.

Disk diffusion method



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Important terms:

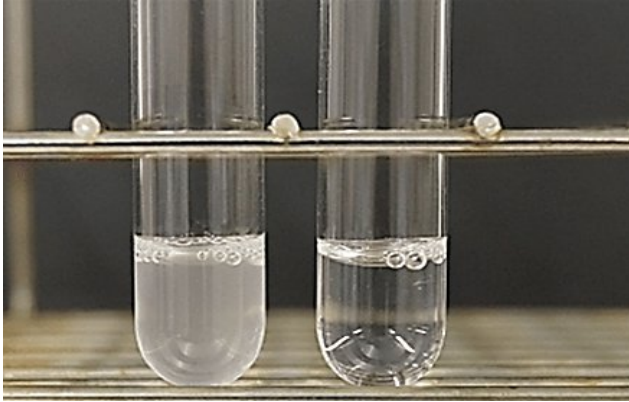
Inoculation

The process of introducing a microorganism OR transferring microbes (like bacteria & fungi) into a culture medium for cultivation. Techniques include streaking, pouring, and stabbing.... etc.

Inoculum الملقح It is small amounts of microorganisms (bacteria, fungi) transferred from a previous culture to a new, fresh medium, often using an inoculation loop .

Bacterial suspension (McFarland standard): where microbial cells in agar or broth (bacteria, fungi) are dispersed throughout a liquid (Buffer, Distilled water or Normal saline), For the purpose of diluting its concentration. Suspensions appear cloudy or turbid .

المعلق البكتيري: هو خليط سائل يحتوي على خلايا بكتيرية موزعة (معلقة) في ماء معقم أو محلول ملحي أو محلول منظم Buffer



Microbial cultures are of two types:

Mixed Culture : two or more types of microorganisms or more than one genus of bacteria are present in culture.

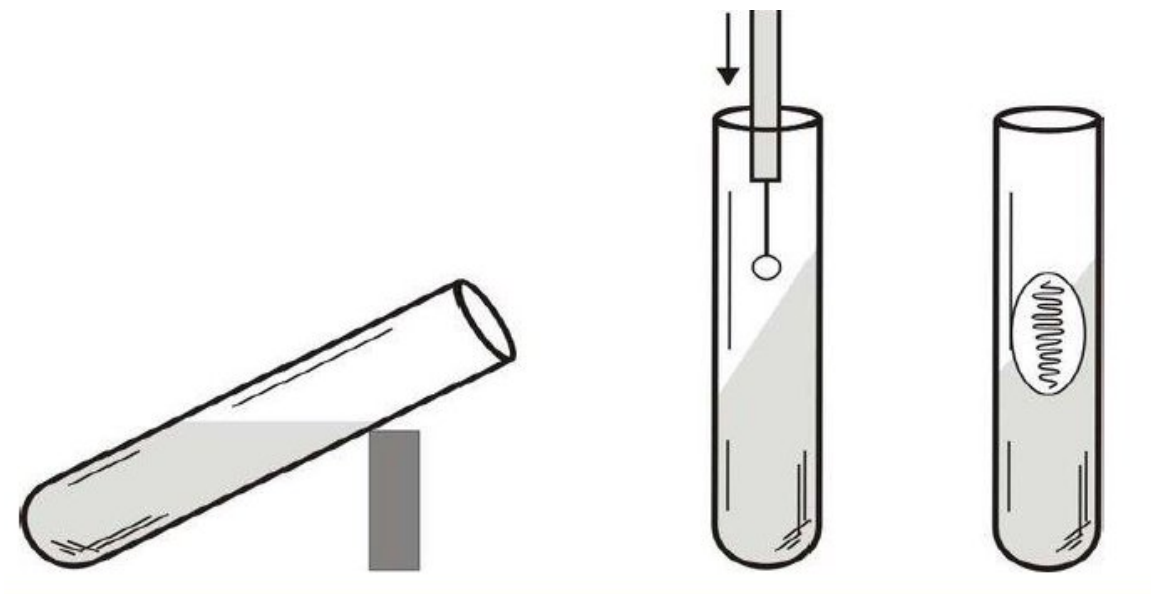
Pure culture : a single species of microorganism or bacteria is present in culture.

A loopful : is the amount of liquid such as broth ,held within the wire loop of an inoculation tool, It's a unit of measurement & Used to pick up and transfer small samples of inoculum or streak microorganisms in microbiology with a standard loop holding approximately 4mm.



2-Stroke culture Method: is a technique used to grow pure bacterial cultures for identification, by streaking bacteria in a zig-zag pattern onto the surface of an agar slant or slope in a test tube.

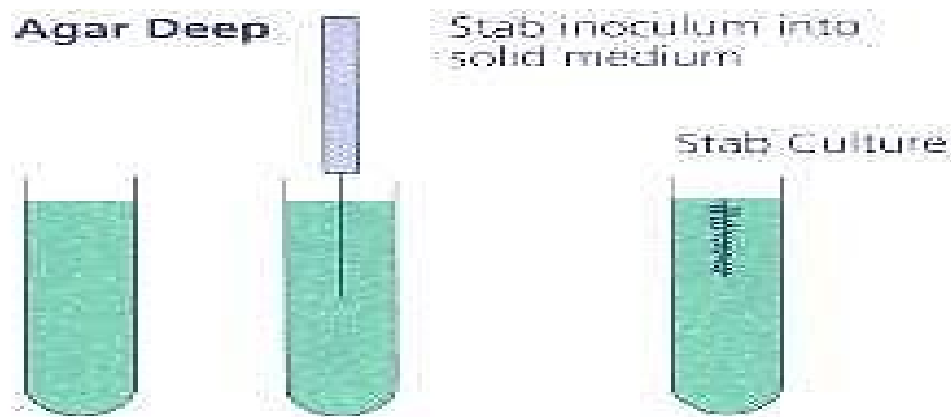
Uses : It is commonly used for maintaining stock cultures.



3- Stab culture method: is used to grow bacteria by stabbing a

straight, sterile needle carrying the microorganism deep into a tube of solid or semi-solid agar medium.

Used for : maintaining stock cultures, testing bacterial motility, and determining oxygen requirements .



4- pour-plate culture : This method **is used for** : counting the number of living bacteria in liquid culture or suspension, by mixing a diluted liquid sample (Either 1.0 ml or 0.1 ml) of dilutions of the bacterial suspension and then pouring the molten agar (40-45 CO) into a Petri dish.

The Colonies grow both on the surface and embedded within the agar.

5- Spread Plate Culture: is method **used to:** enumerate and isolate viable bacteria or fungi from a liquid sample.

A small volume (usually 0.1 mL) of diluted sample is spread over a solid agar surface using a sterile, L-shaped spreader, Allow the sample to absorb into the agar for at least 5–10 minutes .

Then invert the plates and incubate (usually at 37°C for 24 hours).

Q/Why Petri dishes Inverted During Incubation?

Petri dishes are inverted (lid down) during incubation to prevent condensation then falling onto the agar surface, which can cause bacterial colonies to run together **تداخل** and contaminate the culture.

This method ensures obvious, countable colonies and prevents contamination.

