

جامعة الانبار

كلية: الصيدلة

قسم: العلوم المختبرية السريرية

اسم المادة باللغة العربية: الاحياء المجهرية

اسم المادة باللغة الإنكليزية: **microbiology**

المرحلة: الثانية

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عنوان المحاضرة باللغة العربية: الأوساط الزرعية وطرق الحقن

MICROBIAL CULTURE MEDIA & METHODS OF INOCULATION

محتوى المحاضرة

MICROBIAL CULTURE MEDIA & METHODS OF INOCULATION

Media: refers to a mixture of components that will support the growth and cultivation of microorganisms by giving them all the vital nutrients needed for growth in order to cultivate these microorganisms in large numbers for identification and study.

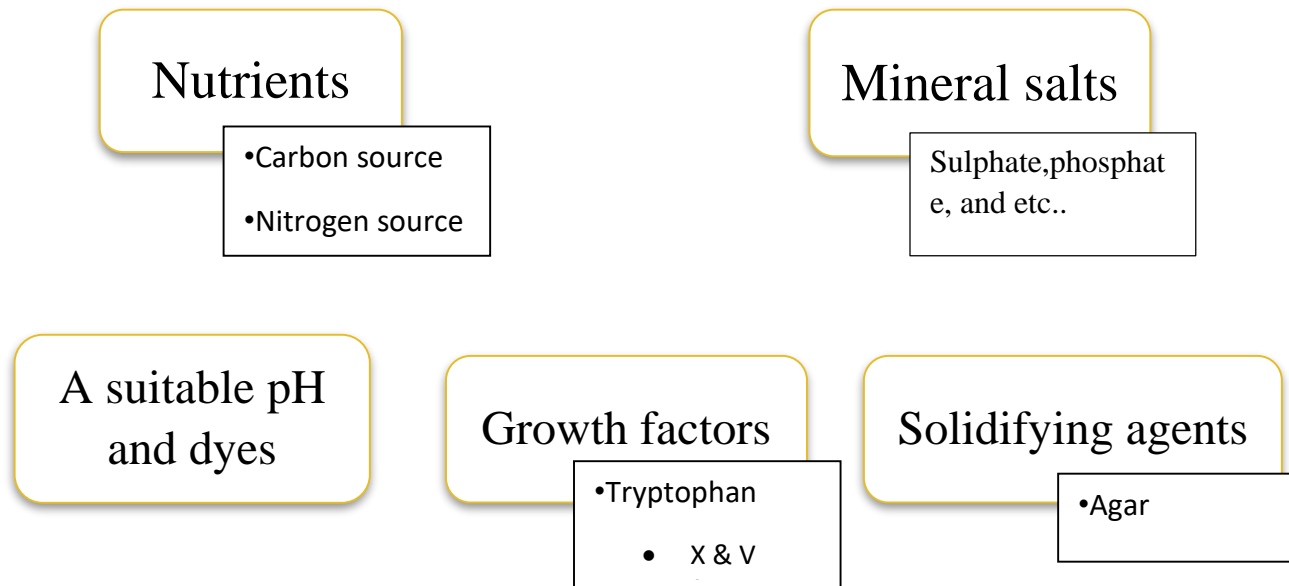
Culture: is the term given to microorganisms that are grown in the lab for the purpose of identifying and studying them using variety media .

Colony: macroscopically visible collection of millions of bacteria originating from a single bacterial cell.

Culture media can be used to:

1. Enrich the number of microorganisms.
2. select for certain microorganism and suppress others.
3. differentiate among different kinds of microorganisms.

Basic requirements of culture media



Classification of culture media

1- classification based on consistency

- Liquid media (No agar)
- Solid media (contains 2% agar)
- Semi-Solid media (contains 0.5% agar)

2- classification based on nutritional components

- Simple media
- Complex media

3- classification based on functional use or application

- Basal media.....Nutrient agar
- Enriched media..... Brain Heart infusion
- selective media..... Mannitol salt agar
- Differential media MacConky agar
- Transport media..... Cary and Blair

Preparation of culture media

The preparation of media from commercial dehydrated products is simple. There are preparation instructions on the label of each bottle of dehydrated medium.

According to the manufacturer's instructions, these media are simply prepared by weighing the required amounts and adding distilled water then melting on magnetic stirrer.



Sterilization of culture media

- Media are sterilized in the autoclave at 121°C for 15' under 15lbs of Pressure
- The components of media that cannot withstand heating at 121°C must first be sterilized by filtration before being added to the media.

CULTURING

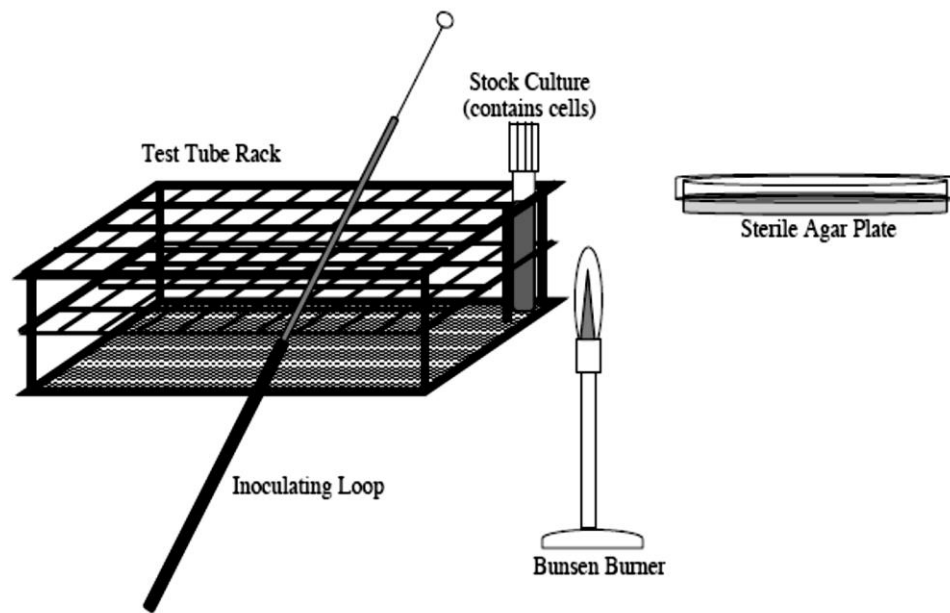
Five basic techniques of culturing

1. Inoculate
2. Incubate
3. Isolation
4. Examination
5. Identification

The purpose of bacterial culture?

- ✓ To isolation of pure culture
- ✓ To obtain information about bacterial biological characteristics

Necessary equipment



INOCULATING TOOLS

To transfer a microbial sample, a variety of instruments may be used; which one is best depends on:

- Sample source and its destination.
- Type of culture medium.



serological pipette



Pasteur pipette



needle



loop



cotton swab



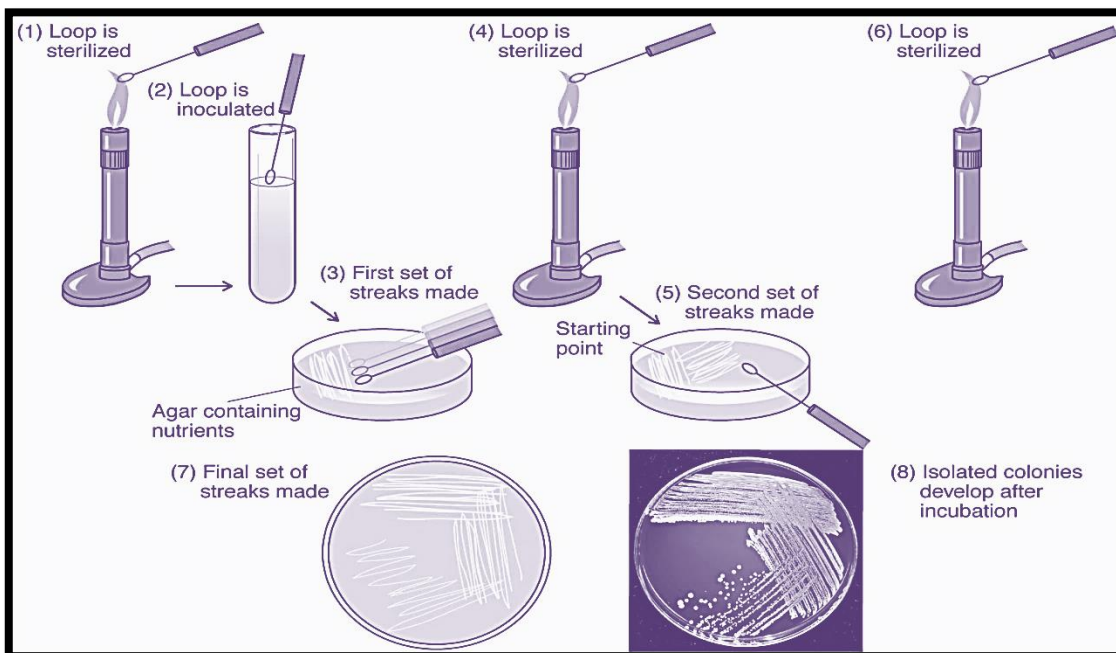
spreader

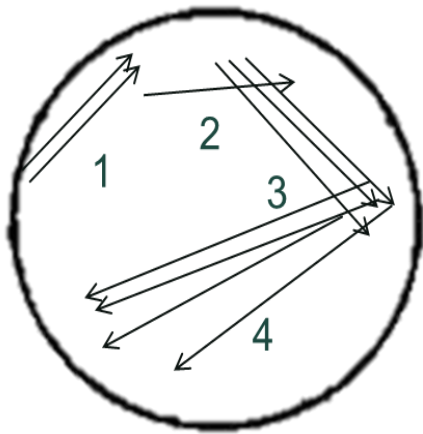
METHODS OF INOCULATION

- Streaking
- Spreading
- Pour Plate
- LAWN culture method
- Stabbing

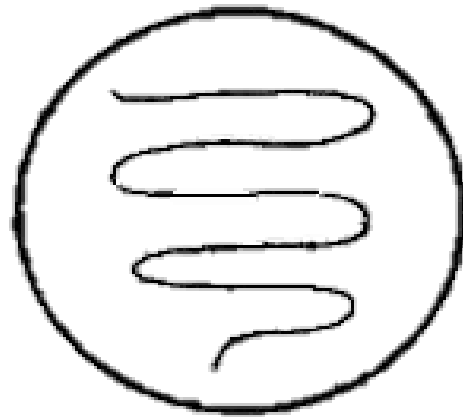
❖ Streaking

- Used to isolation of bacteria in pure culture from clinical specimens.
- Loop is used with this method.
- One loopful of the specimen (inoculum) is transferred onto the surface of plate.
- The inoculum is then distributed thinly over the plate by streaking it with a loop.





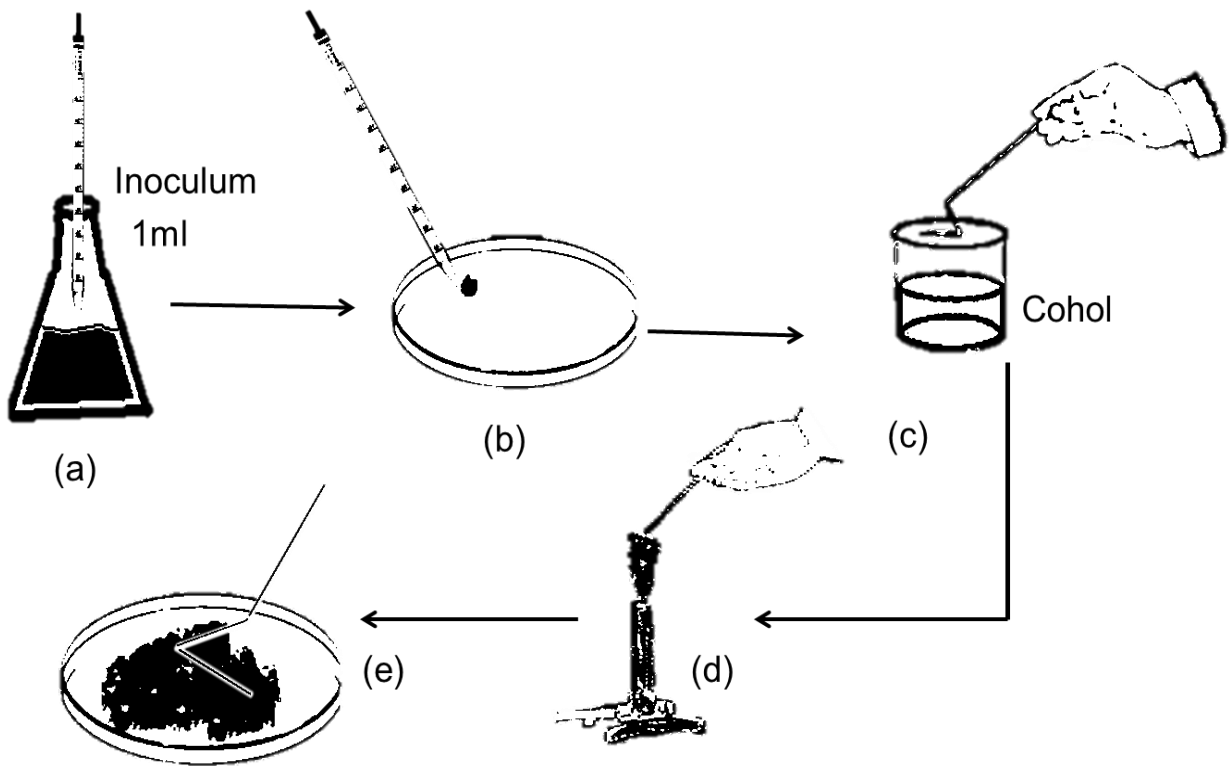
Streaking



Zigzag

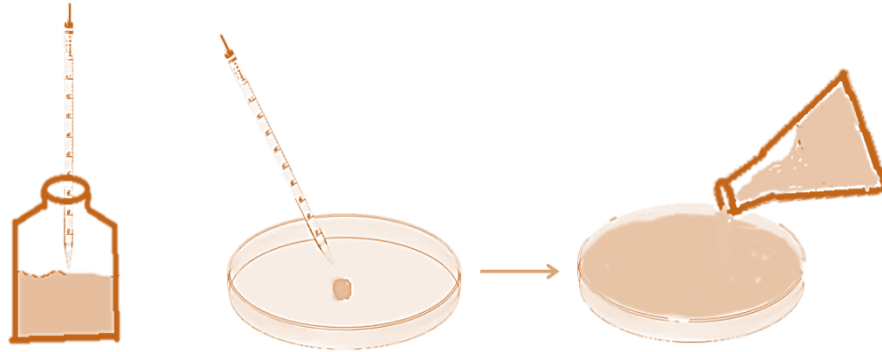
❖ **SPREADING**

Using a sterile spreader to spread liquid sample on the Petri plate as follow:



❖ POUR PLATE

- Using a sterile pipette to transfer liquid sample in the Petri plate before pour the media.
- To distribute the inoculum, rotate the plate in a circular motion.



Put 1ml of sample
in the plate

After pouring the medium
mix with circular motion

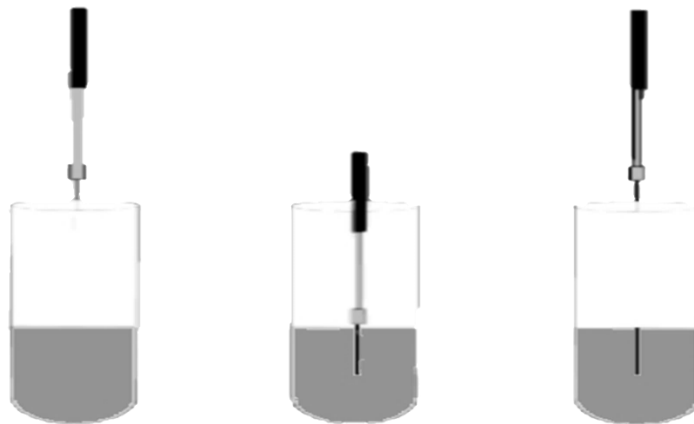
❖ LAWN culture method

- Provides a uniform surface growth of the bacterium.
- Used for antibiotic sensitivity testing.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium using cotton swab.



❖ Stabbing

Stab tubes (deeps): are tubes of agar medium which are inoculated by "stabbing" the inoculum into the agar using sterile needle.



- Notice that the inoculating needle is moved into the tube without touching the walls of the tube, and the needle penetrates medium to its depth.

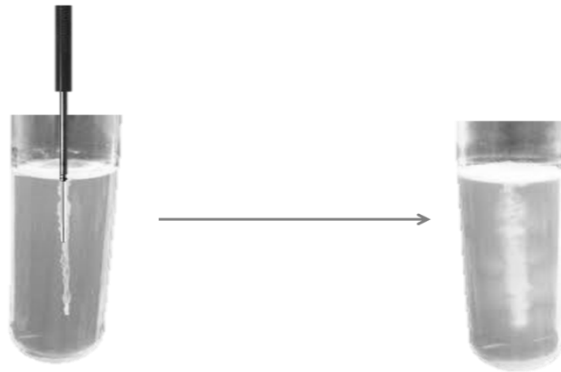
- **HOW TO INOCULATE A SLANT?**

- Slant tubes: are tubes containing a nutrient medium plus agar.
- The medium has been allowed to solidify at an angle in order to get a flat inoculating surface.
- A loop is using to streak the surface of the slant.



- **HOW TO INOCULATE A SEMI SOLID MEDIA?**

A needle uses to inoculate a stab medium, for example mannitol motility medium.



- **HOW TO INOCULATE A LIQUID MEDIUM?**

Broths and other liquid media are inoculated using a sterile wire loop, or Pasteur pipette depending on whether the inoculum is colonial growth or a fluid culture or specimen.

FORMS OF GROWTH IN BROTH MEDIA

Nutrient broth After incubation, growth (development of many cells from a few cells) may be observed as one or a combination of three forms

a. Pellicle: A mass of organisms is floating on top of the broth.



b. Turbidity: The organisms appear as a general cloudiness throughout the broth.



c. Sediment: A mass of organisms appears as a deposit at the bottom of the tube.



LABELING OF INOCULATED MEDIA

Using a marker pen, label inoculated media with the date and the patient's number. Always label the base of a culture plate. A slope should be labeled on the underside of the media. A stab culture should be labeled above the level of the agar.