MICROBIOLOGICAL CULTURE MEDIA & METHODS OF INOCULATION

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CULTURE MEDIA

- ***Media***: is the term given to the combination of ingredients that will support the growth of microorganisms by providing all the essential nutrients required for the growth in order to cultivate these microorganisms in large numbers to study them.
- The survival and growth of microorganisms depend on available nutrients and a favorable growth environment.

Why?

Culture media can be used to:

- enrich the number of microorganism.
- select for certain microorganism and suppress others.
- differentiate among different kinds of microorganisms.

Basic requirements of culture media

Nutrients

- Carbon source
- Nitrogen source

Mineral salts

Sulphate, Phosphates, and etc..

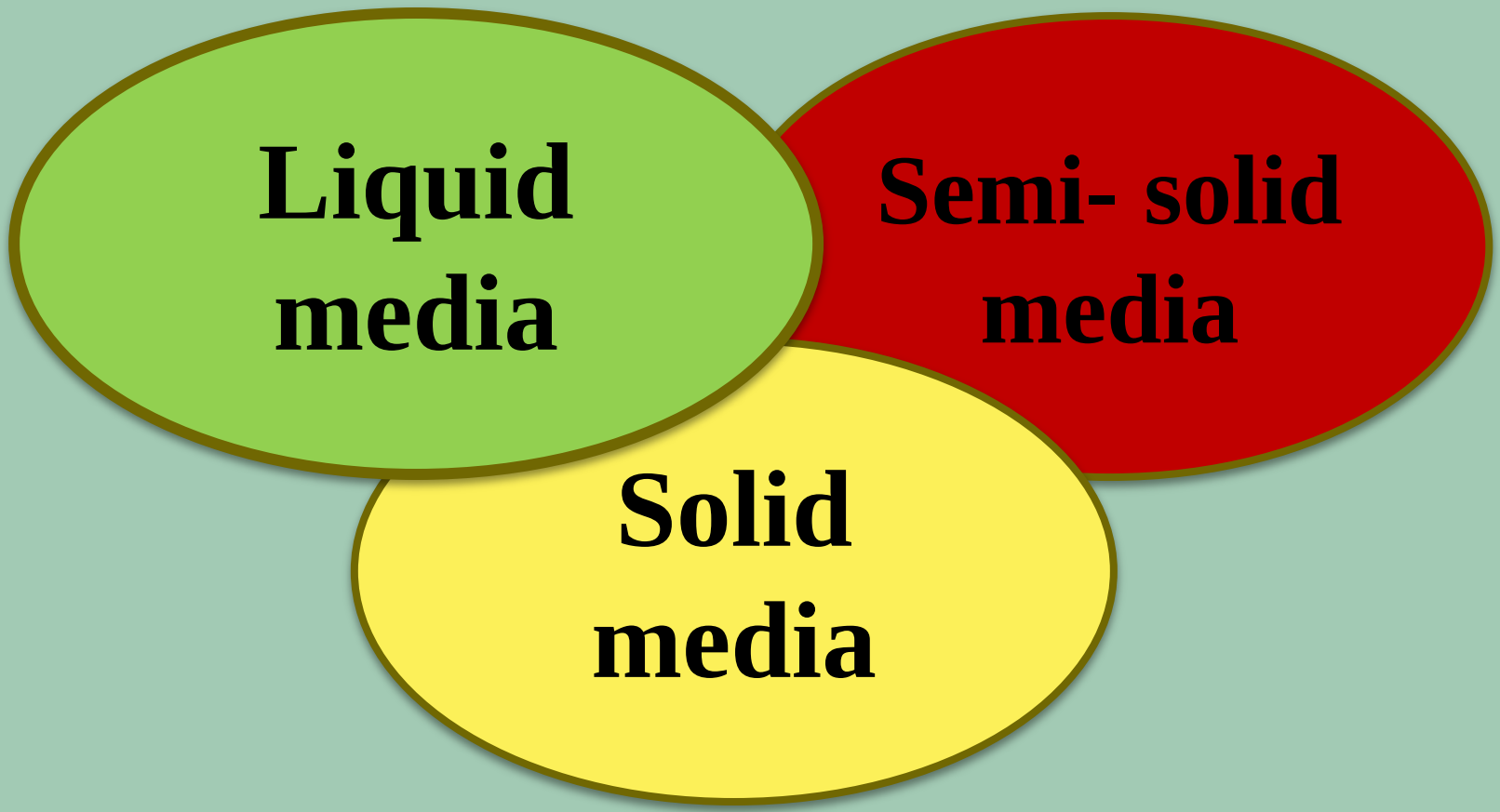
A suitable pH and dyes

Growth factors

Solidifying agents

Classification of culture media

1- classification based on consistency



Classification of culture media

2- classification based on nutritional components

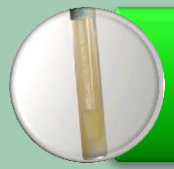
Simple media

Complex media

Classification of culture 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. Each bottle of dehydrated medium has instructions for preparation on its label.
- Media are sterilized in the autoclave at 121^0C for 15 minutes under 15 bar of Pressure

CULTURING

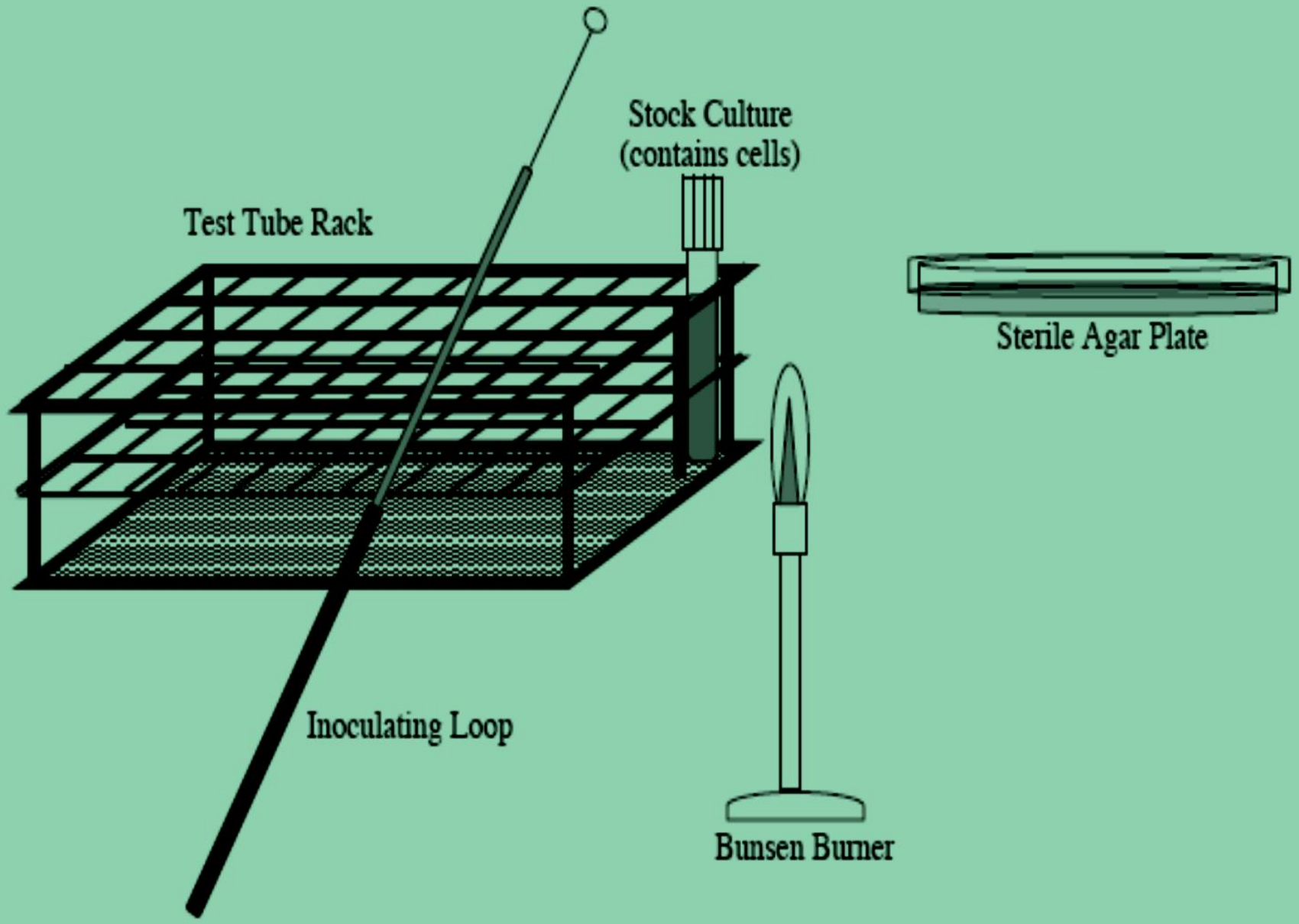
- Five basic techniques of culturing

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

Why?

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

Necessary equipment



INOCULATING TOOLS

There are several different instruments may be used to transfer a microbial sample, the choice of which depends on:

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

serological pipette

Pasteur pipette

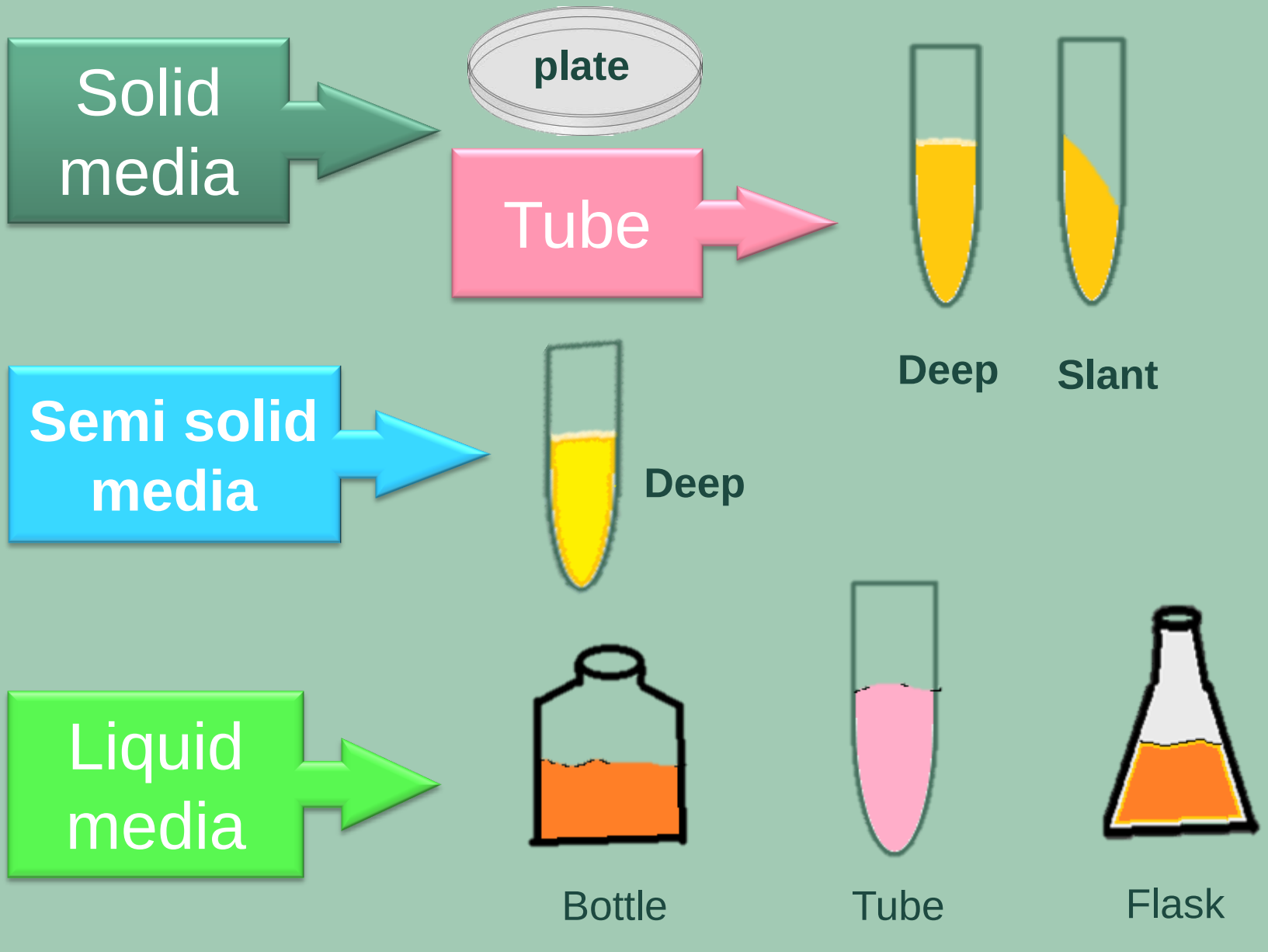
needle

loop

cotton swab

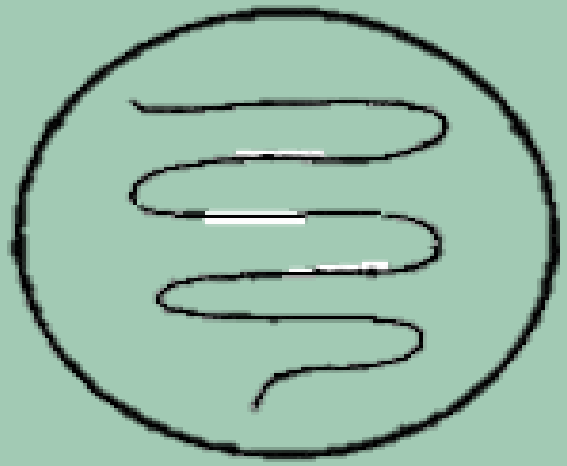
spreader

METHODS OF INOCULATION

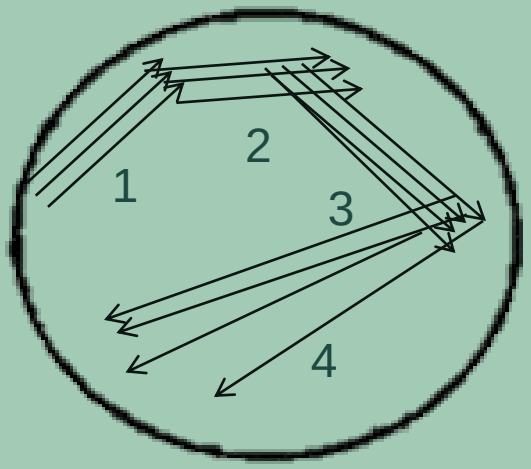


HOW TO INOCULATE A CULTURE PLATE

- Plate provide large surface for isolation and observation of colonies.
- Using a loop or a swab streak your sample on the Petri plate as follow:

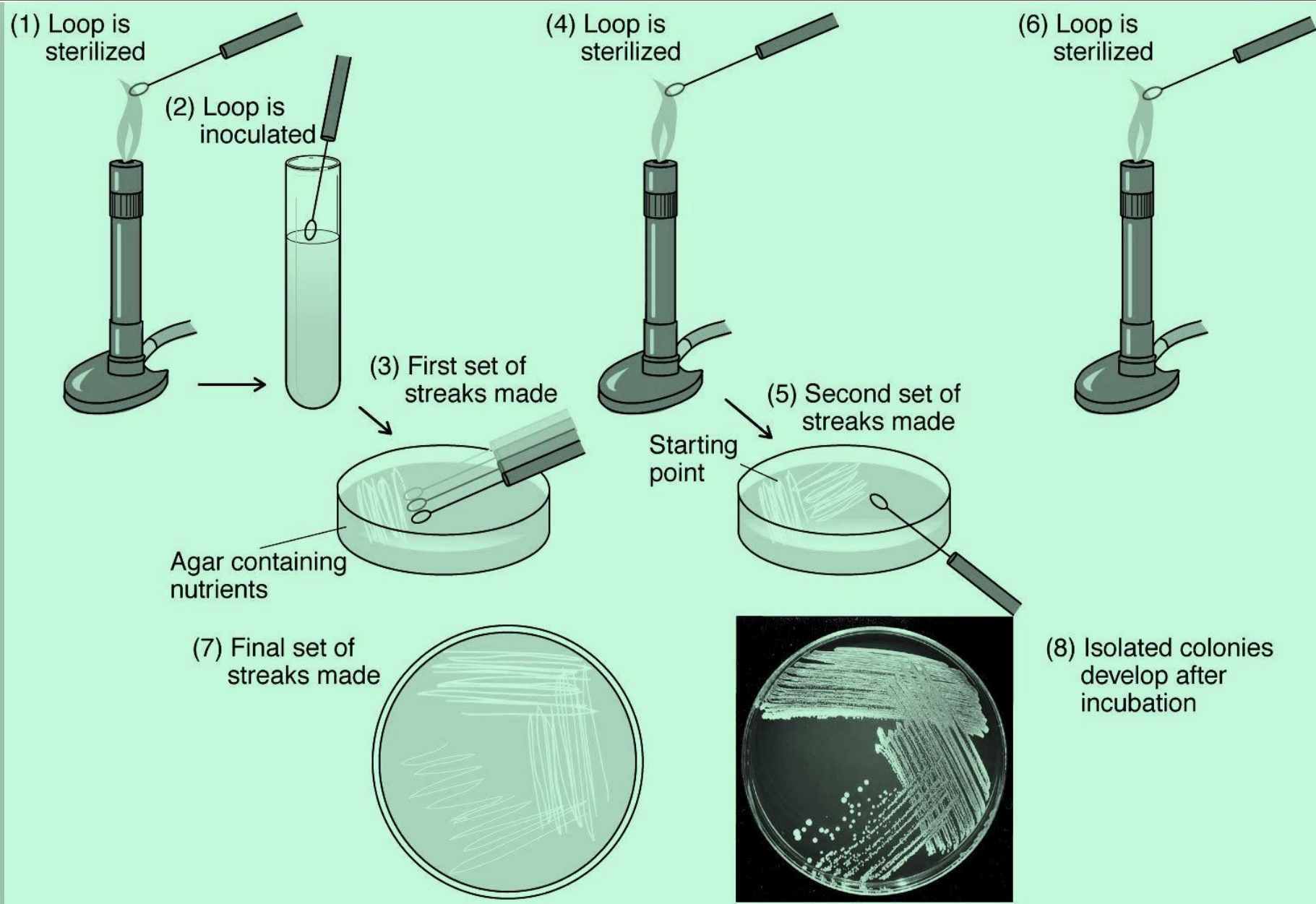


1. Zigzag

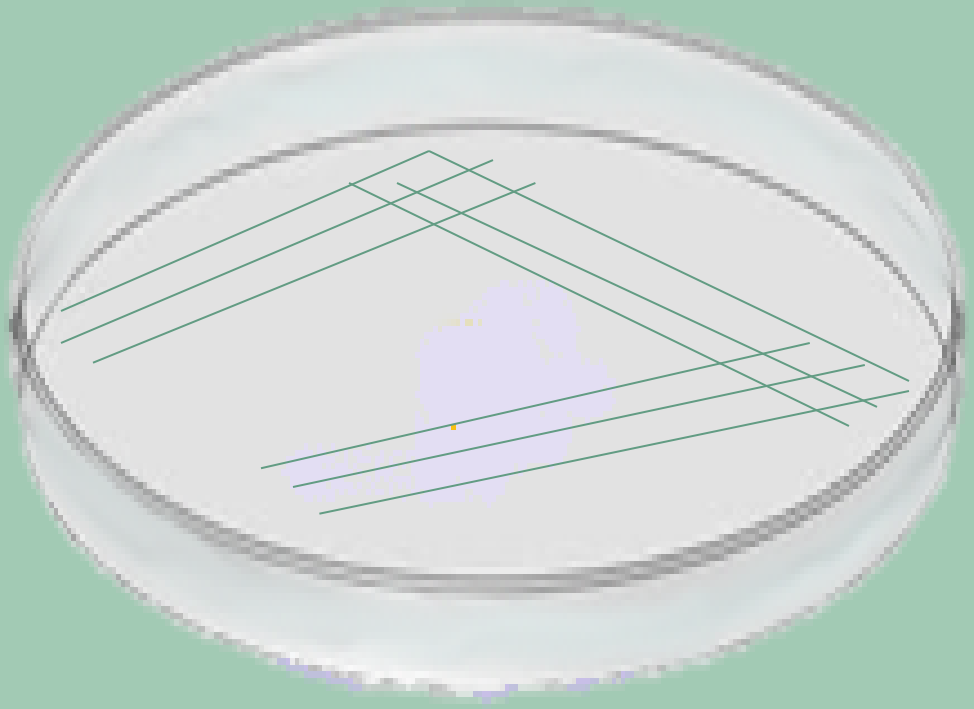


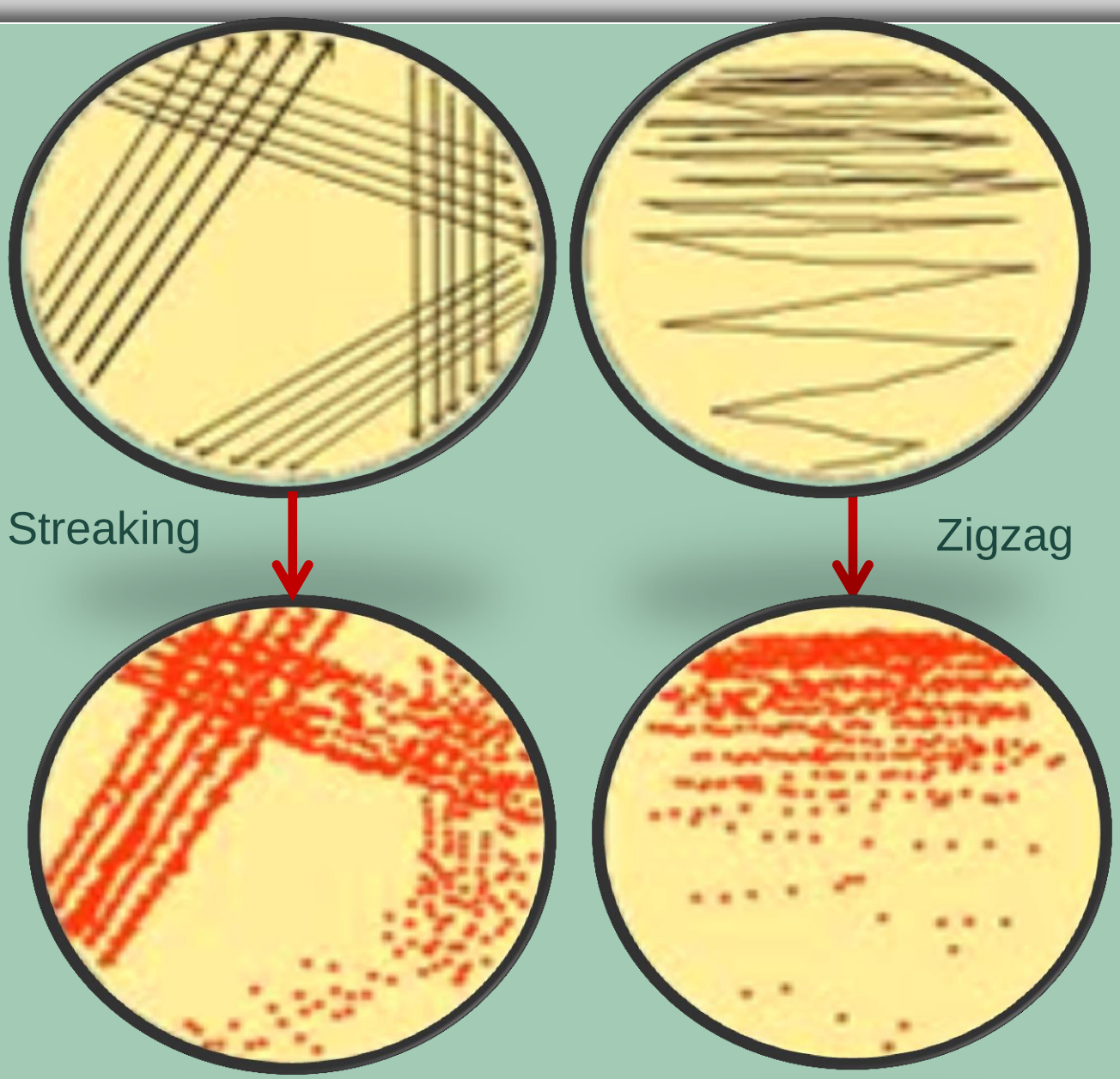
2. streaking

STREAK PLATE METHOD



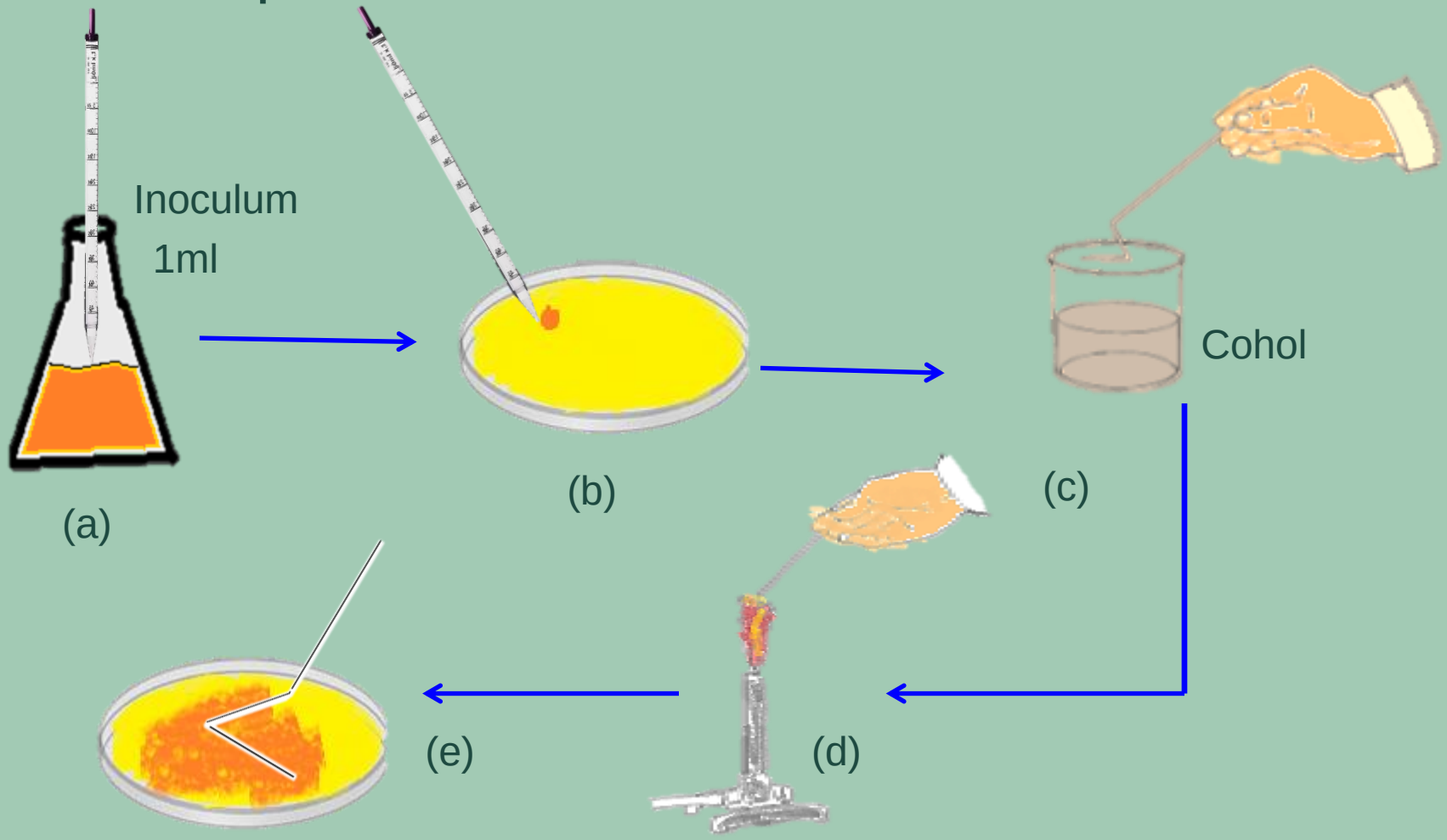
streaking




Streaking
Zigzag

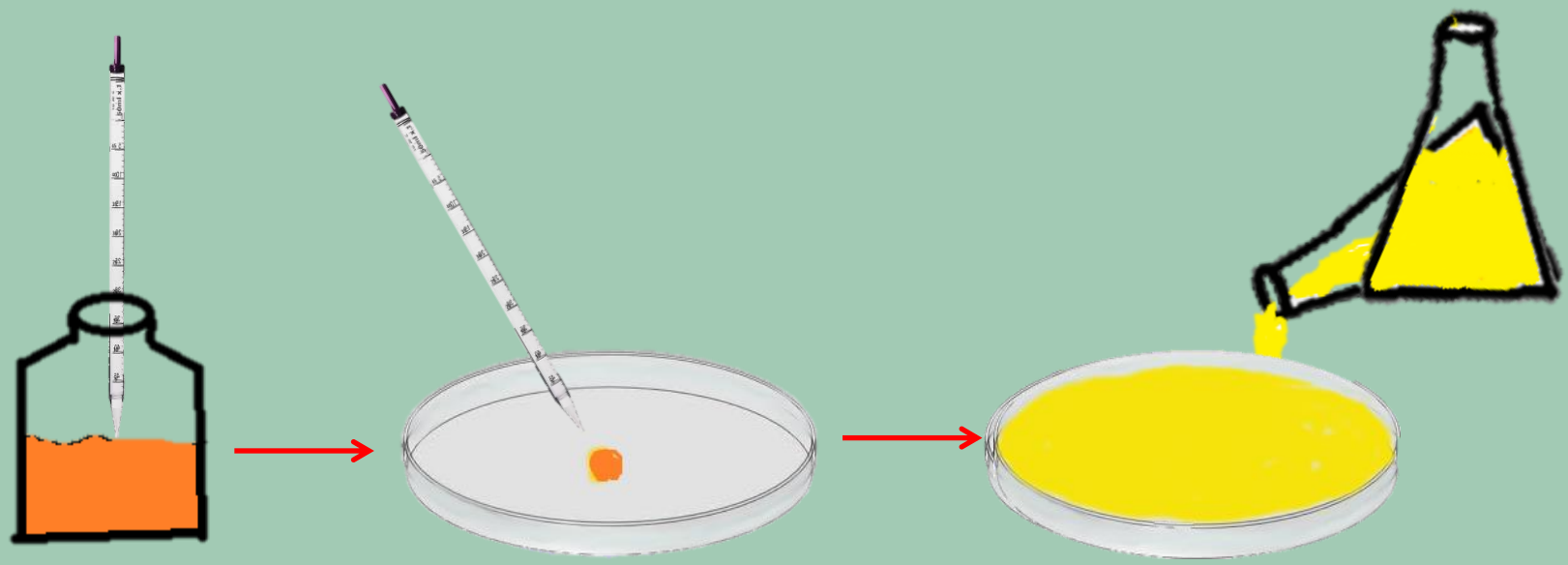
SPREADING

- 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 as follow:

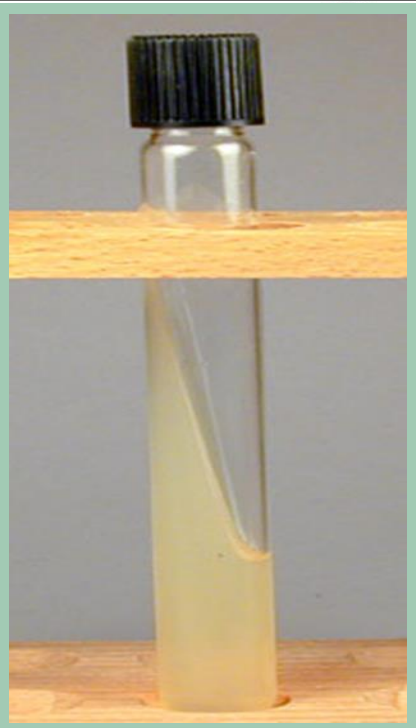


Put 1ml of sample in the plate

After pouring the medium mix with circular motion

HOW TO INOCULATE A SLANT

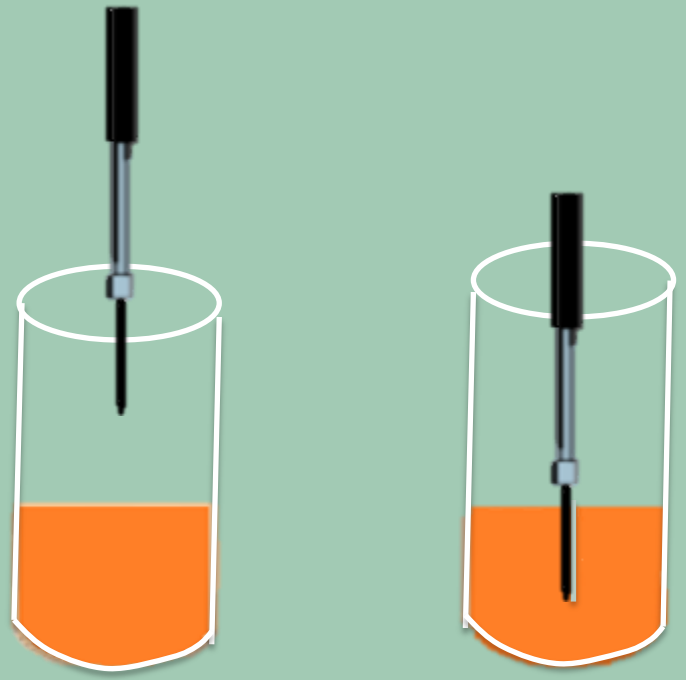
Slant tubes: are tubes containing a nutrient medium plus agar. The medium has been allowed to solidify at an angle in order to get a flat inoculating surface .



A loop is using to streak the surface of the slant.

HOW TO INOCULATE A STAB TUBE

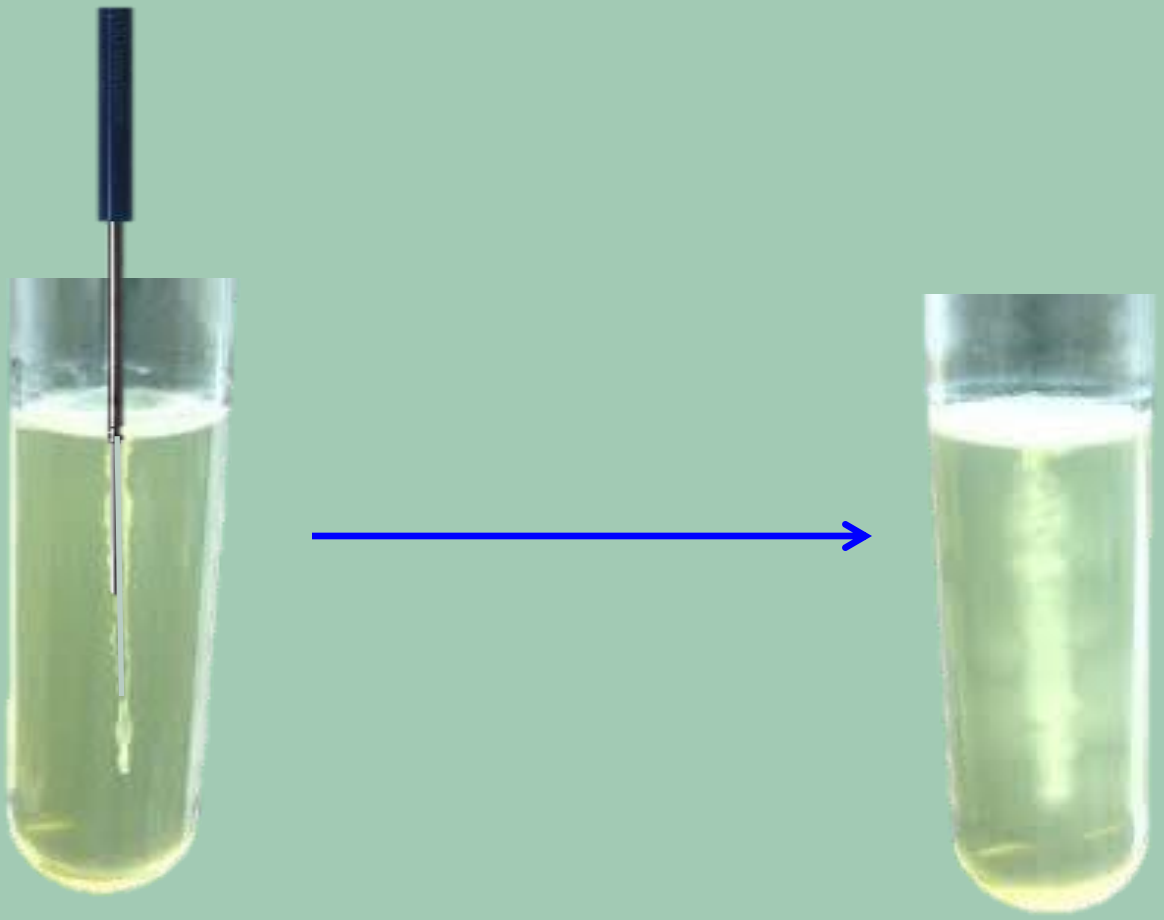
Stab tubes (deeps): are tubes of agar medium which are inoculated by "stabbing" the inoculum into the agar using sterile needle .



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 SEMI SOLID MEDIA

A needle use 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.

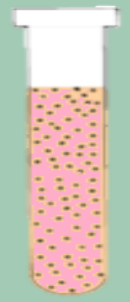
FORMS OF GROWTH IN BROTH MEDIA

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.

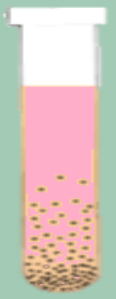
Growth Layered at Surface only

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



Growth turbid And diffuse throughout

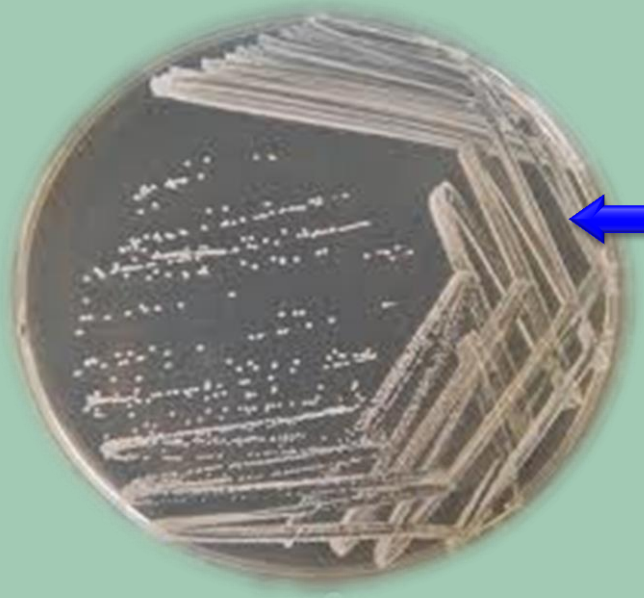
c. **Sediment**: A mass of organisms appears as a deposit at the bottom of the tube.



Growth Sediment at bottom only

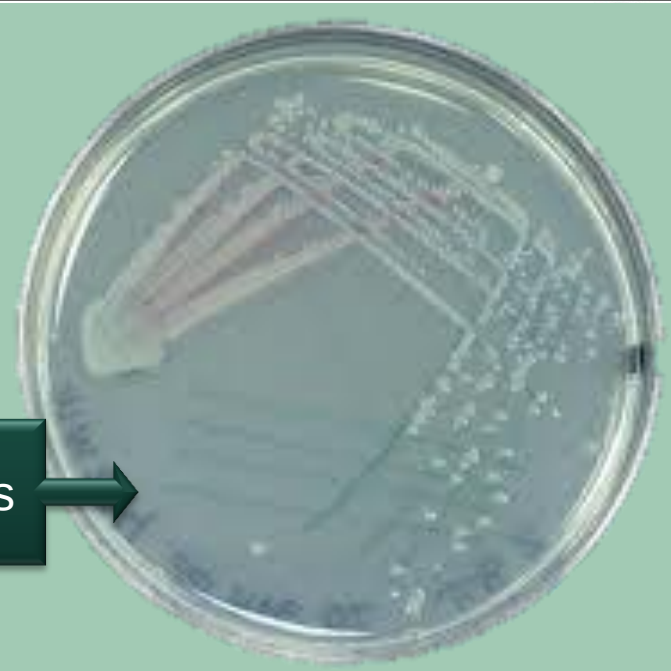
LABELING OF INOCULATED MEDIA

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.



streptococcus

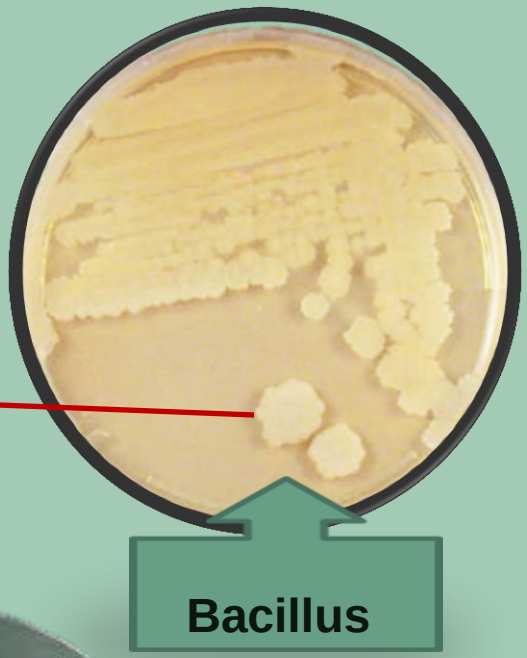
staphylococcus



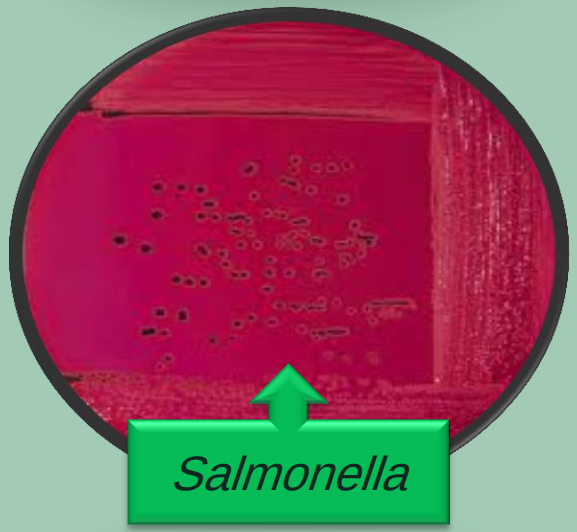
klebsiella

E. coli

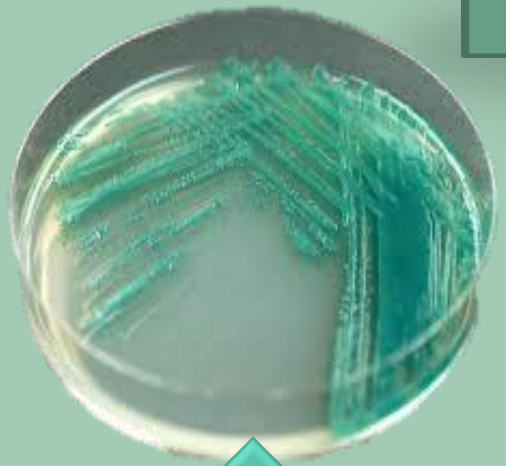
Swarming of *Proteus*



Bacillus



Salmonella



Psedomonas

End of lecture

Thank you for listening