Lab 2 Bacterial culture

Introduction

Laboratory diagnosis of infectious diseases can be accomplished by a series of procedures which are:

- Specimen collection.
- Microscopic examination.
- **Culture and isolation of the organism in pure form.**
- Biochemical tests.
- Serological tests.
- Molecular identification.
- Antibiotic sensitivity.

Bacterial culture

A method that allows the multiplication of bacterial cells in or on a culture medium under controlled laboratory conditions.

Bacteria are grown in the laboratory for the following reasons:

- To isolate them from pathological specimens for identification and diagnosis “Culture of the microorganism is the **Gold Standard** method of diagnosis”.
- To study their characteristic features.
- To determine their antibiotic sensitivity.
- To prepare antigens, toxins and vaccines.

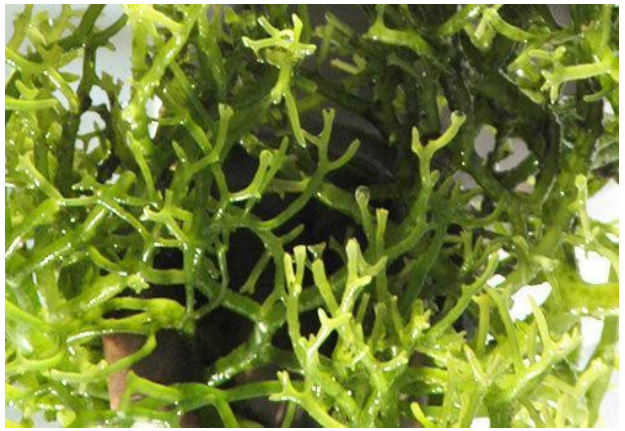
Culture media

The nutrient preparation on which microorganism is grown in the laboratory.

A culture medium is essentially composed of basic elements, to which added different growth factors that will be specific to each bacterium and necessary for their growth.

Main components of culture media:

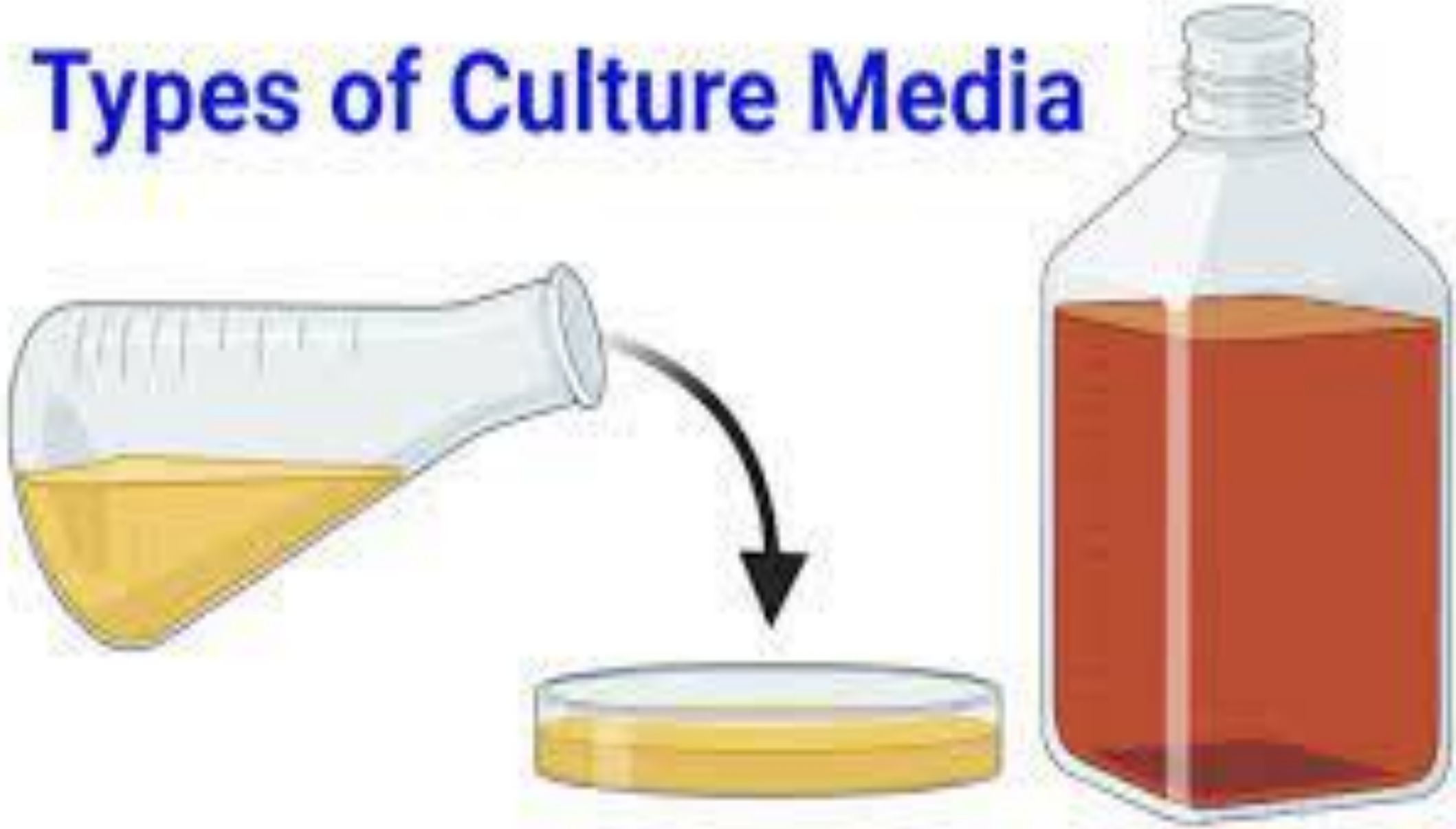
- Water
- Peptone
- Meat extract
- Yeast extract
- Mineral salts
- Carbohydrates
- **Agar:** “Inert polysaccharide from sea weed”,



Non-nutritive and **not metabolized** by microorganism,

It is solidifying agent which dissolves at 90-100 °C and solidify at 40 °C.

Types of Culture Media



According to physical state:

- Liquid media.
- Semi-solid media.
- Solid media.

A) Liquid media:

- **Examples:** Peptone water, Broth, Blood culture bottle.
- **Uses:**

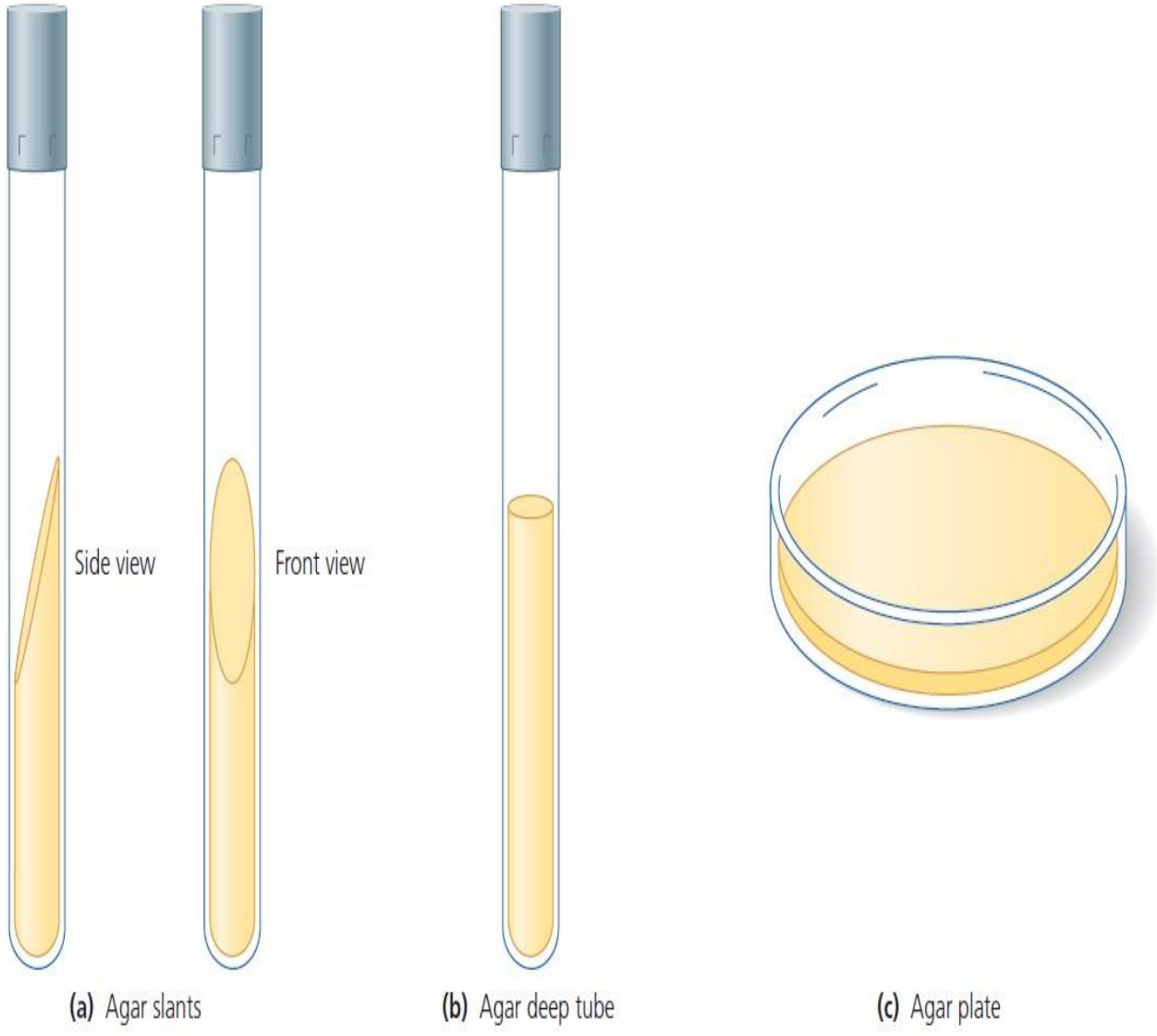
- For biochemical reactions.
- For blood culture.
- To test large volume of sample as water.
- As enrichment media.
- Transport media.

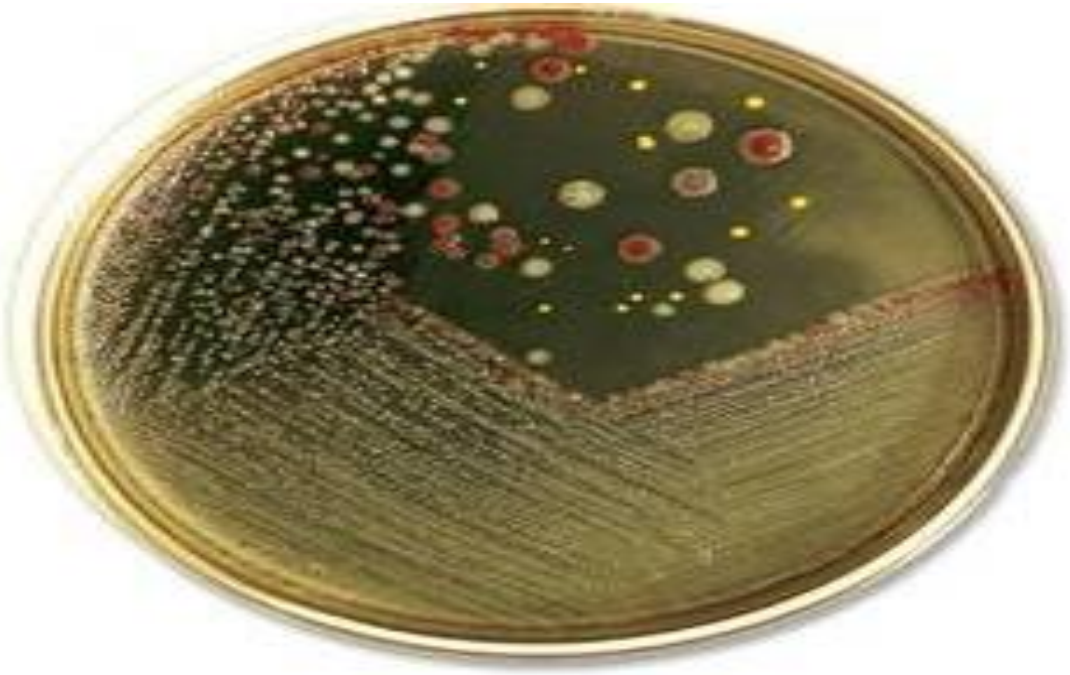
Blood culture bottle

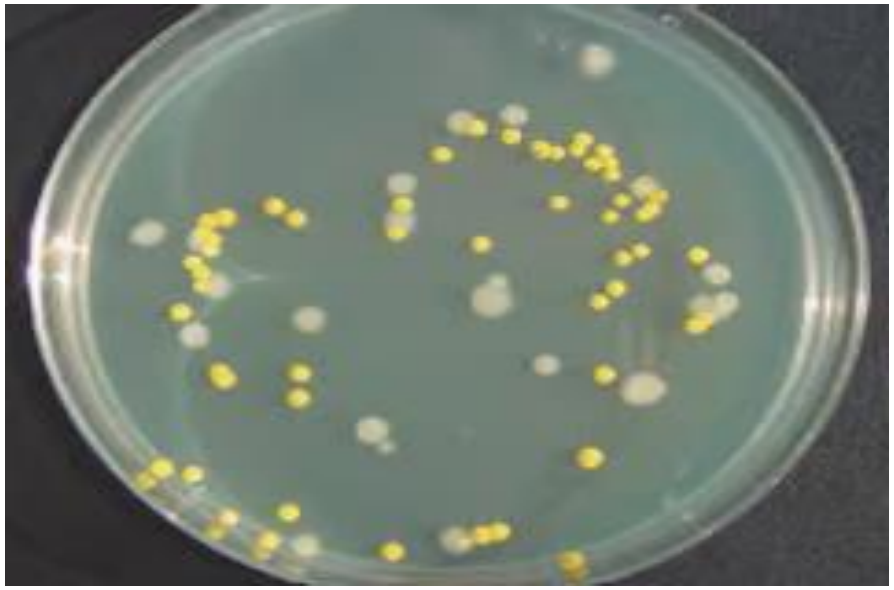
Nutrient broth

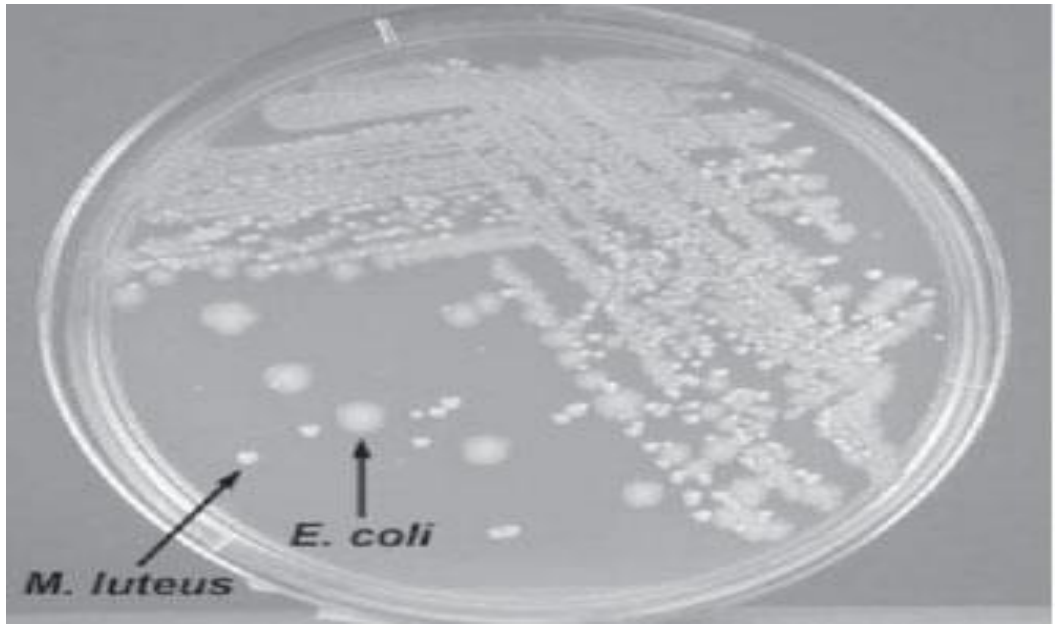
B) Solid media:

- **Contain 1.5-2% agar.**
- **Forms:**
  - Plate “petri dishes”.
  - Slope tubes.
  - Deep tubes.
- **Uses:**
  - Isolation of pure colonies.
  - Description of bacterial colonies: morphology, pigmentation, hemolysis,...







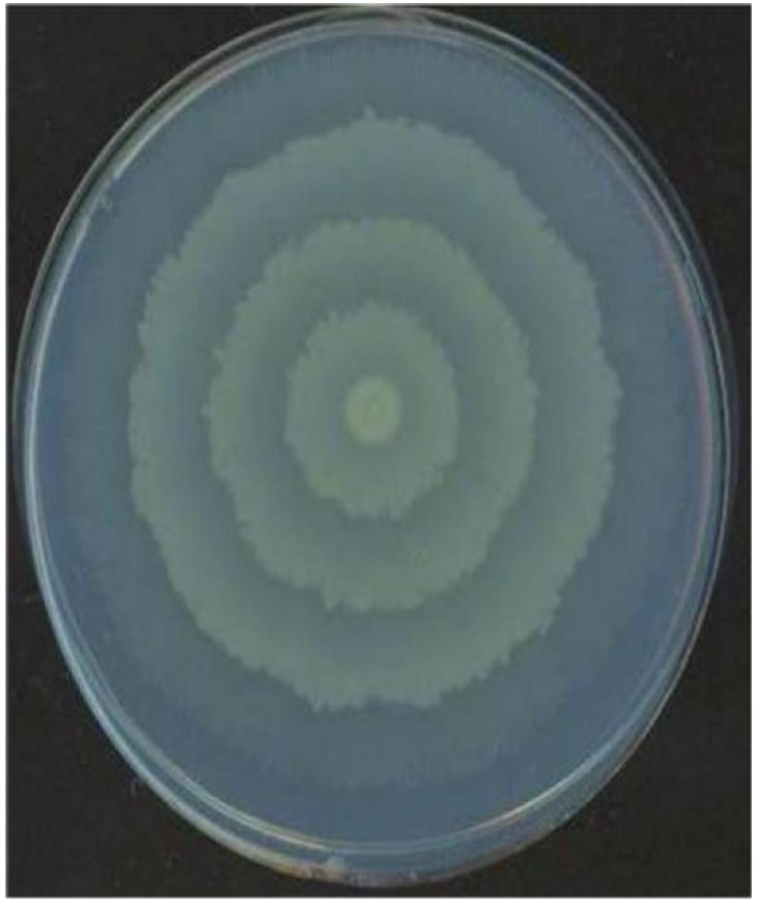

E. coli
M. luteus

C) Semi-solid media:

- **Prepared by:** Decreasing agar concentration.

"Contain 0.5 % agar"

- **Uses:** Motility testing.



According to applications:

- Simple media
- Enriched media
- Selective media
- Differential (Indicator) media
- Enrichment media
- Transport media
- Media for anaerobic cultivation

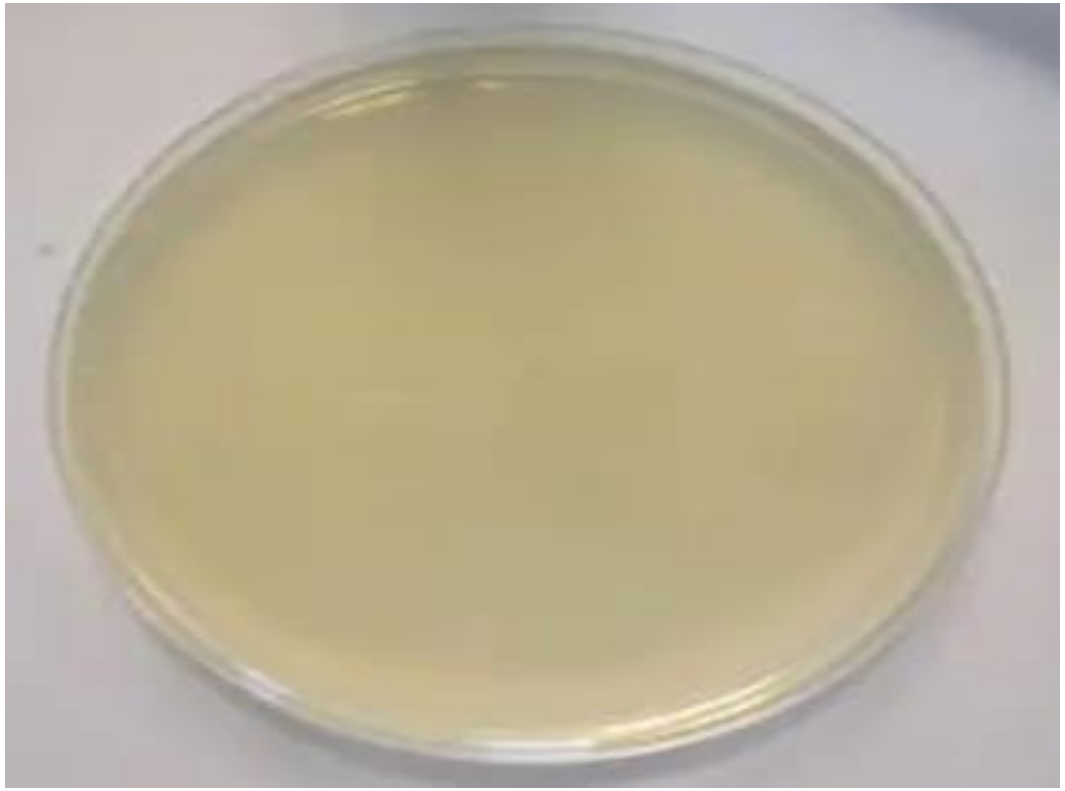
Simple Media

➢ **Used for:**

- Support growth of microorganisms that do not have special nutritional requirement.
- As a base for other media.

➢ **Examples:**

- Peptone water
- Nutrient broth
- Nutrient agar



Nutrient agar

Enriched media

➢**Used for:**

Cultivation of fastidious organisms that need high nutritive substances for growth e.g. blood, serum or egg.

➢**Examples:**

- Blood agar
- Chocolate agar
  =(heated blood agar)
- Loeffler's serum
- Dorset's egg medium

Blood agar

Chocolate agar

Selective media

Contain some chemicals, dyes or antibiotic which inhibit the growth of certain organisms & allow the growth of others.

Examples:

- Lowenstein-Jensen medium (L.J)

  (for Mycobacterium tuberculosis).
- Blood tellurite agar (for Diphtheria).
- Thayer-Martin medium (for Neisseria).
- Thiosulphate Citrate Bile Sucrose (TCBS) (for vibrio).
- Mannitol Salt Agar (for staphylococci).
- Salmonella-Shigella Agar.

Lowenstein-Jensen

Differential or Indicator media

Media that contain certain ingredient or indicator that permit the differentiation between the organisms according to their effects on the media or color change.

Examples:

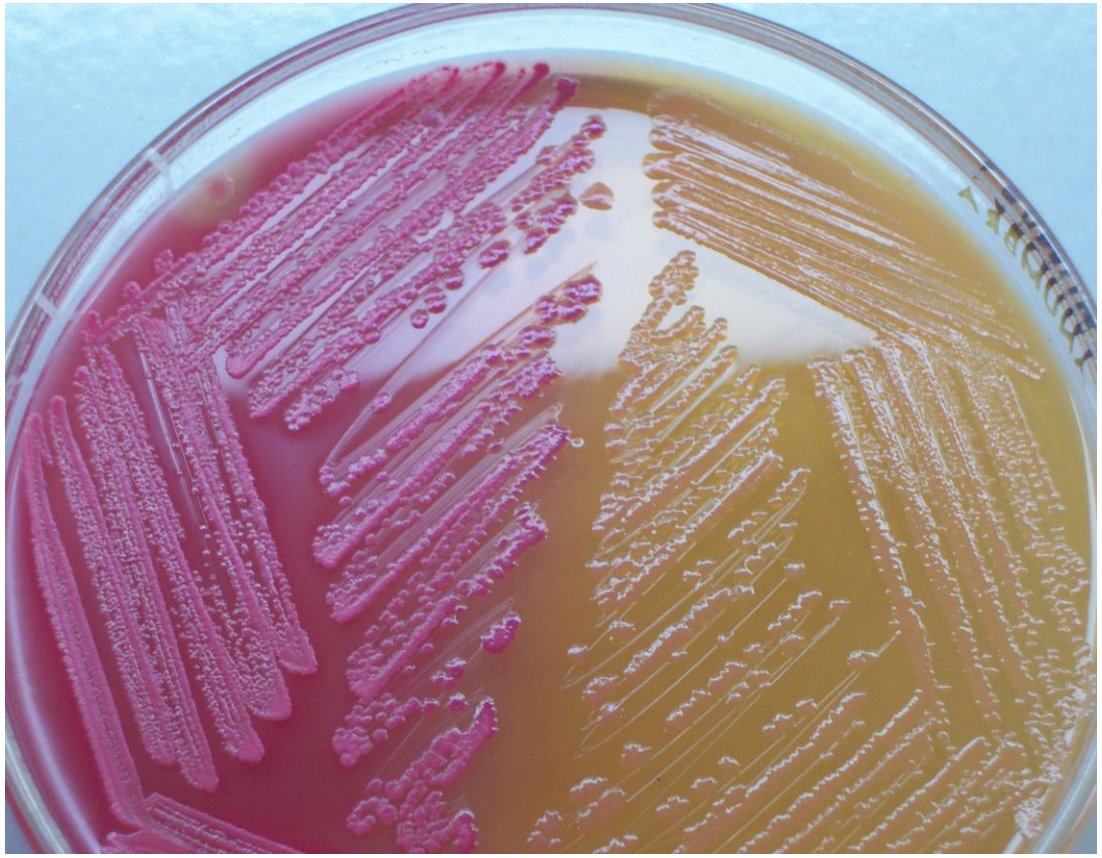
- MacConkey agar
- TCBS agar
- Mannitol salt agar
- Salmonella-Shigella Agar

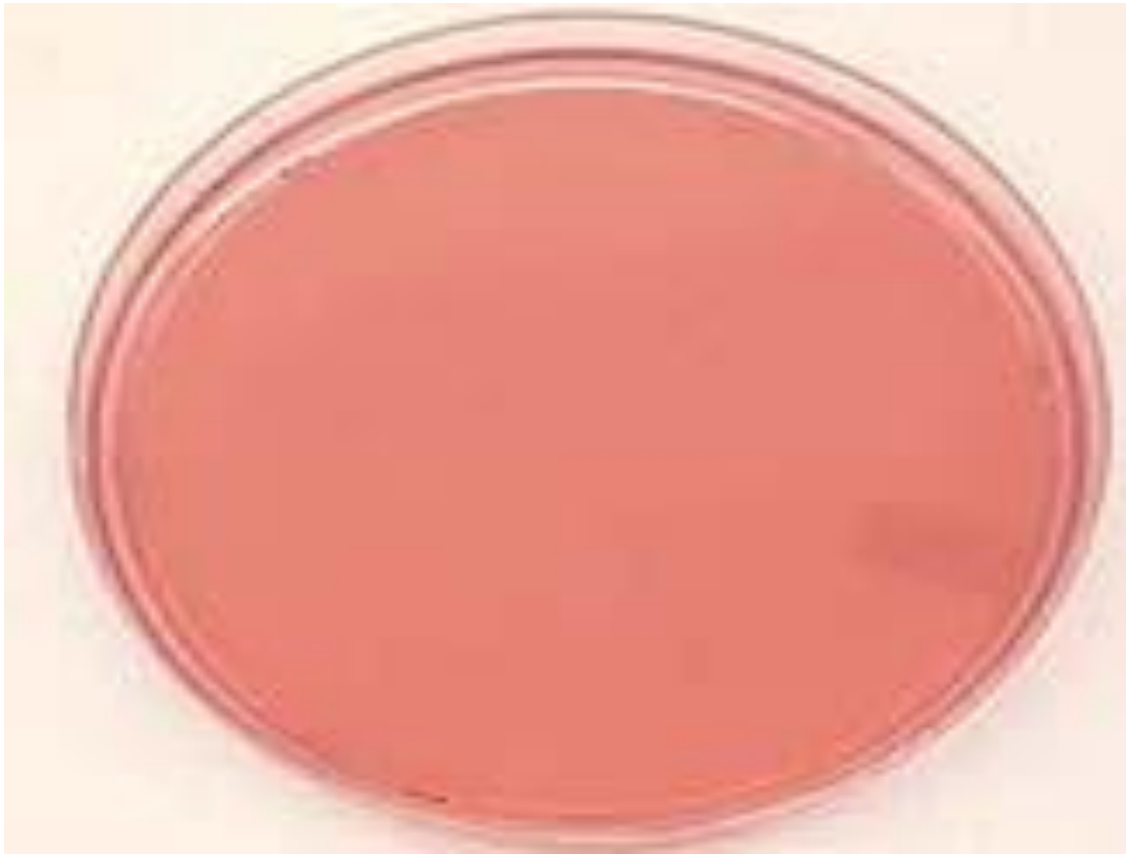
Indicator

- Blood agar

MacConkey agar

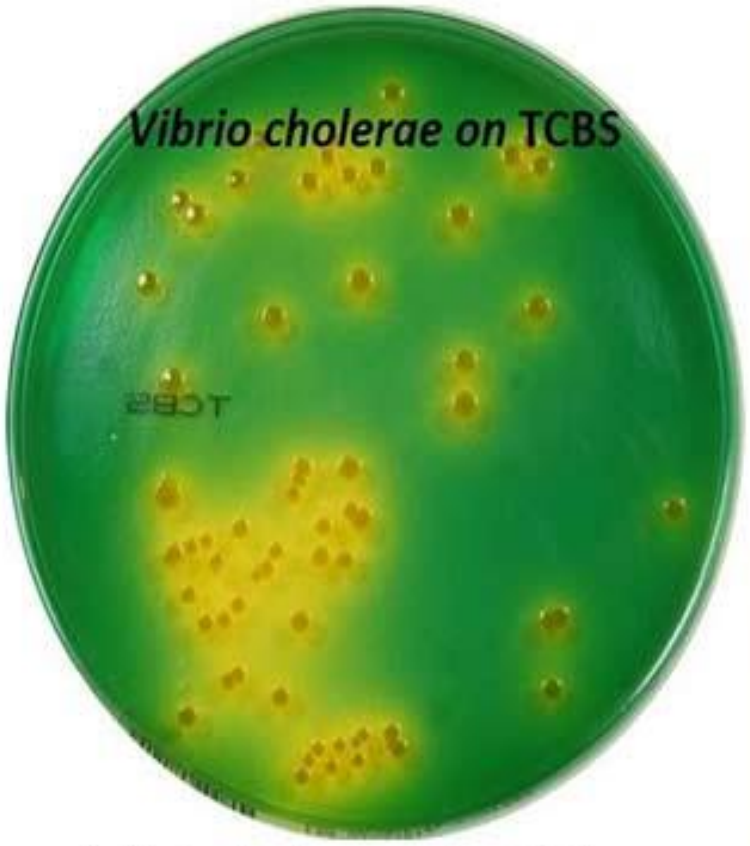
- Contain lactose & **neutral red** indicator.
- Differentiate between Lactose fermenters (pink colonies) e.g E.coli, and Non lactose fermenters (pale yellow colonies) e.g Salmonella

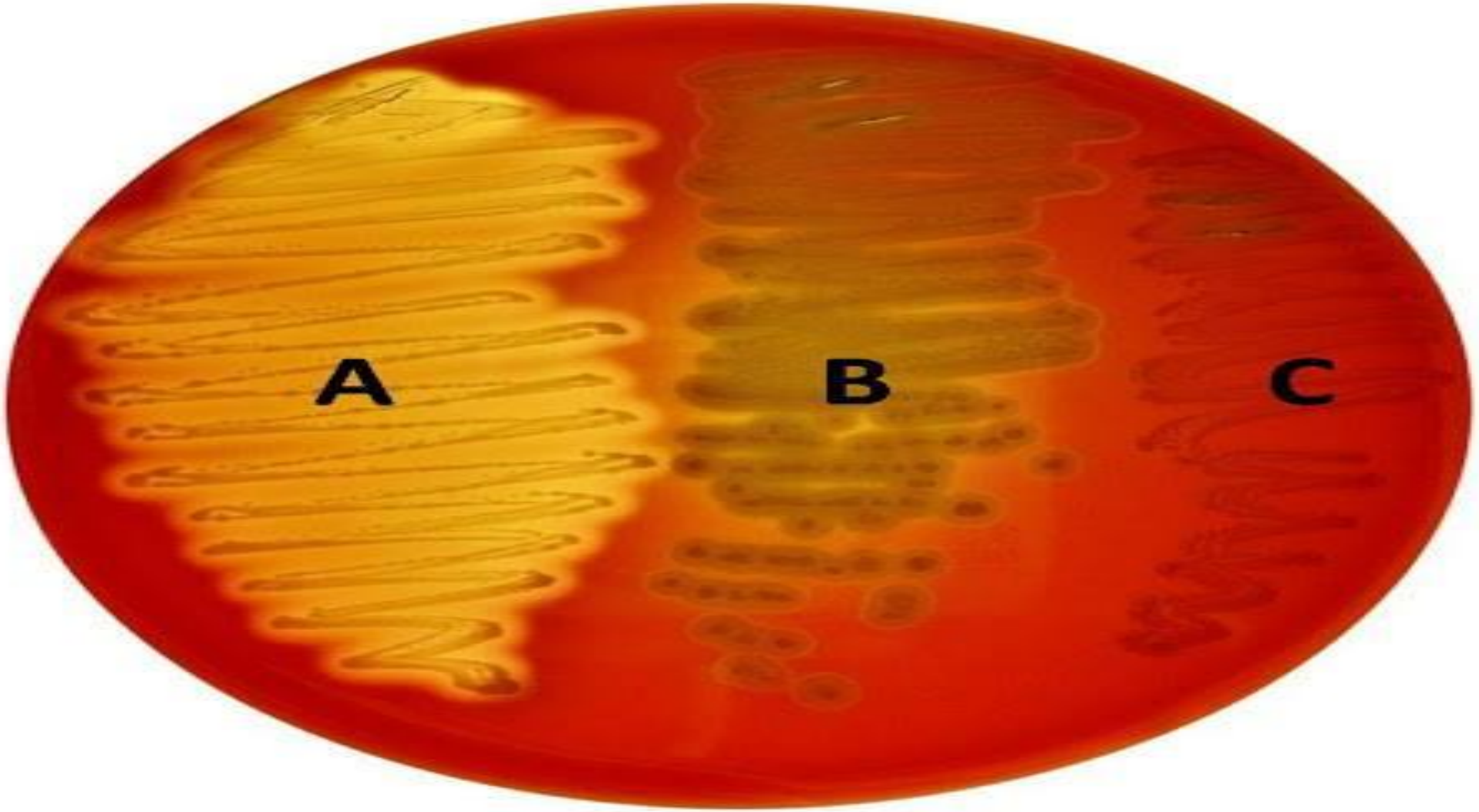




Thiosulphate Citrate Bile Sucrose (TCBS)

- Contain sucrose & **bromothymol blue** indicator.
- Differentiate between vibrio cholera (yellow colonies) and non-cholera vibrio (green colonies).





A: β-hemolysis
Streptococcus pyogenes

B: α-hemolysis
Streptococcus viridans

C: γ (No) hemolysis
Streptococcus faecalis

Enrichment media

Fluid containing some substances which stimulate the growth of some organisms on expense of the unwanted organisms.

Examples:

- Selenite broth & Tetrathionate broth (for isolation of Shigella & Salmonella from stool).
- Alkaline peptone water (for isolation of Vibrio cholera).

Transport media

Transport media for microorganisms are a non-nutritive, balanced, buffered medium that provides a controlled environment to **preserve the viability** of bacteria during transport **without allowing them to multiply,** when specimens cannot be cultured immediately after collection.

Examples:

- Amies Transport Medium.
- Stuart’s medium.

Anaerobic culture

Anaerobic cultivation is essential for bacteria that cannot grow in the presence of O2 e.g. Clostridium & Bacteroids.

Robertson cooked meat medium

The following methods are used for anaerobiosis:

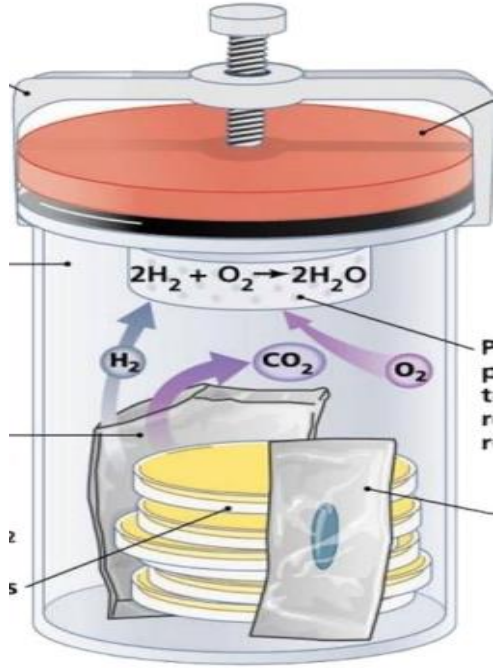
➢Use of media containing reducing compounds as:

- **Robertson cooked meat medium.**
- **Thioglycolate broth.**

➢Replacement of oxygen by hydrogen using **Gas–pak jar.**

Anaerobic Gas Pak System:

- It is Gas Generating Systems in which **hydrogen** is generated inside the jar by placing a special Gas Pak envelope commercially prepared.
- The presence of **catalyst** in the jar allows the hydrogen released to combine with oxygen in jar to give strictly anaerobic condition.



Bacterial culture techniques

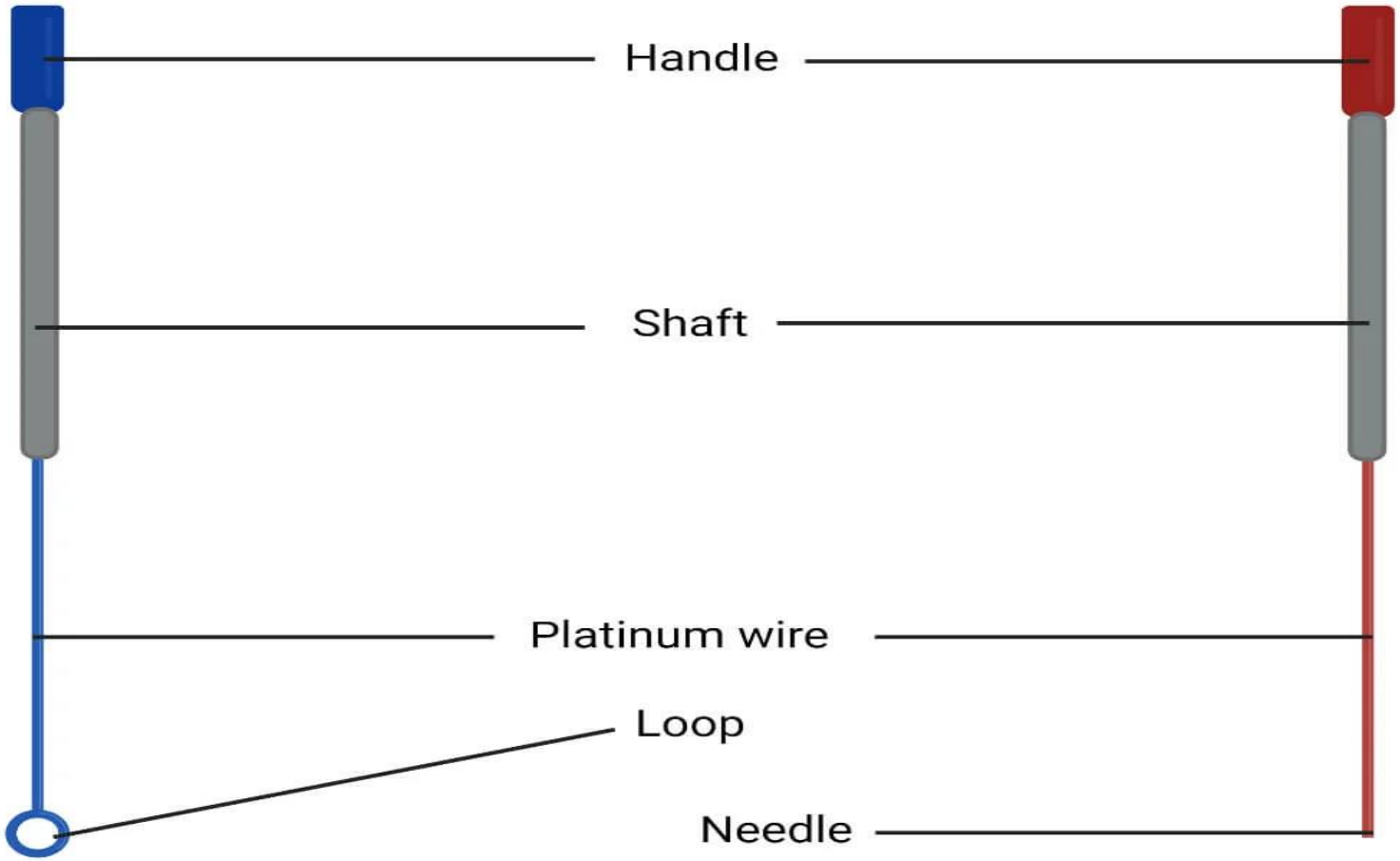
A) Inoculation: – introduction of a sample into a culture media (By sterile loop and using aseptic technique).

- **Inoculation of fluid media**
- **Inoculation of solid media:**
  - **Streak culture**
  - **Slant culture**
  - **Stab culture**
  - **Lawn culture**

B) Incubation: under conditions that allow growth.

(i.e. suitable temperature, humidity, CO2%,..)

Parts of Inoculating Loops and Needles



Inoculating Loop

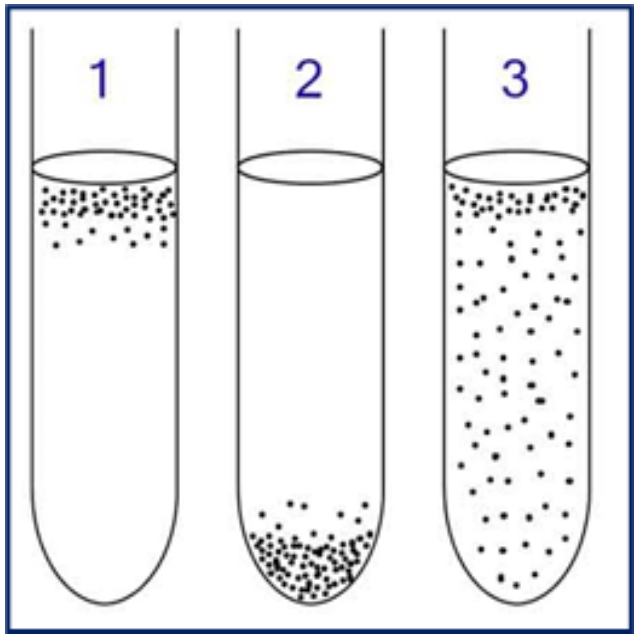
Inoculating Needle

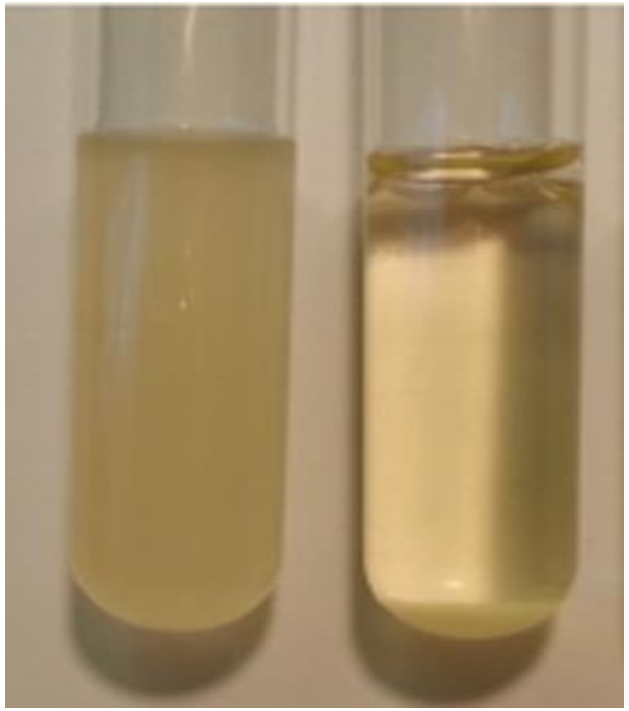
Inoculation of fluid media:

By adding a portion of the specimen to the medium.

Identification of bacterial growth on fluid media:

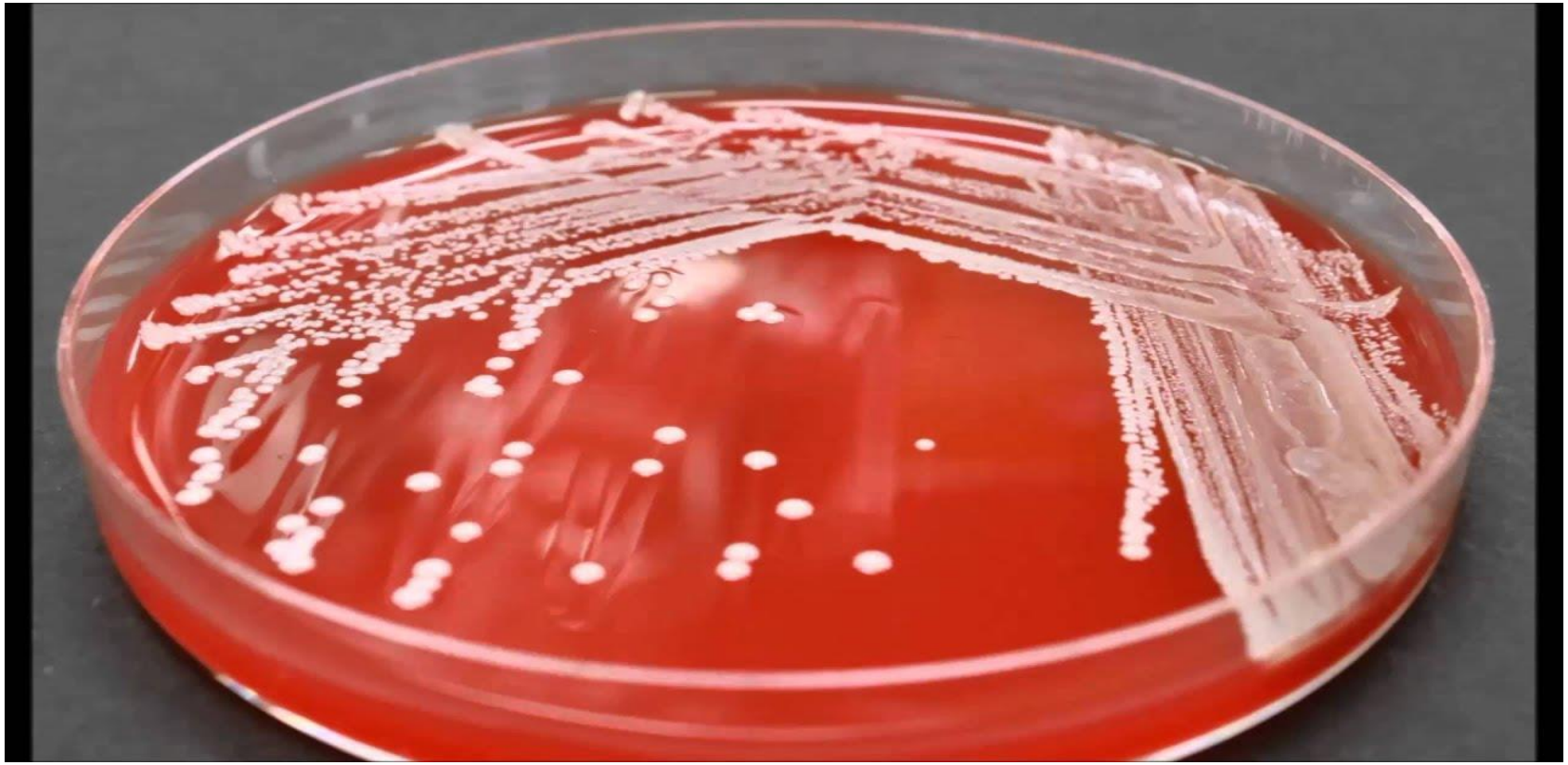
1. **General turbidity** if the bacteria are facultative anaerobes.
2. **Surface pellicle** if the bacteria are aerobic.
3. **Deposits** in the bottom of the tube in case of anaerobes.



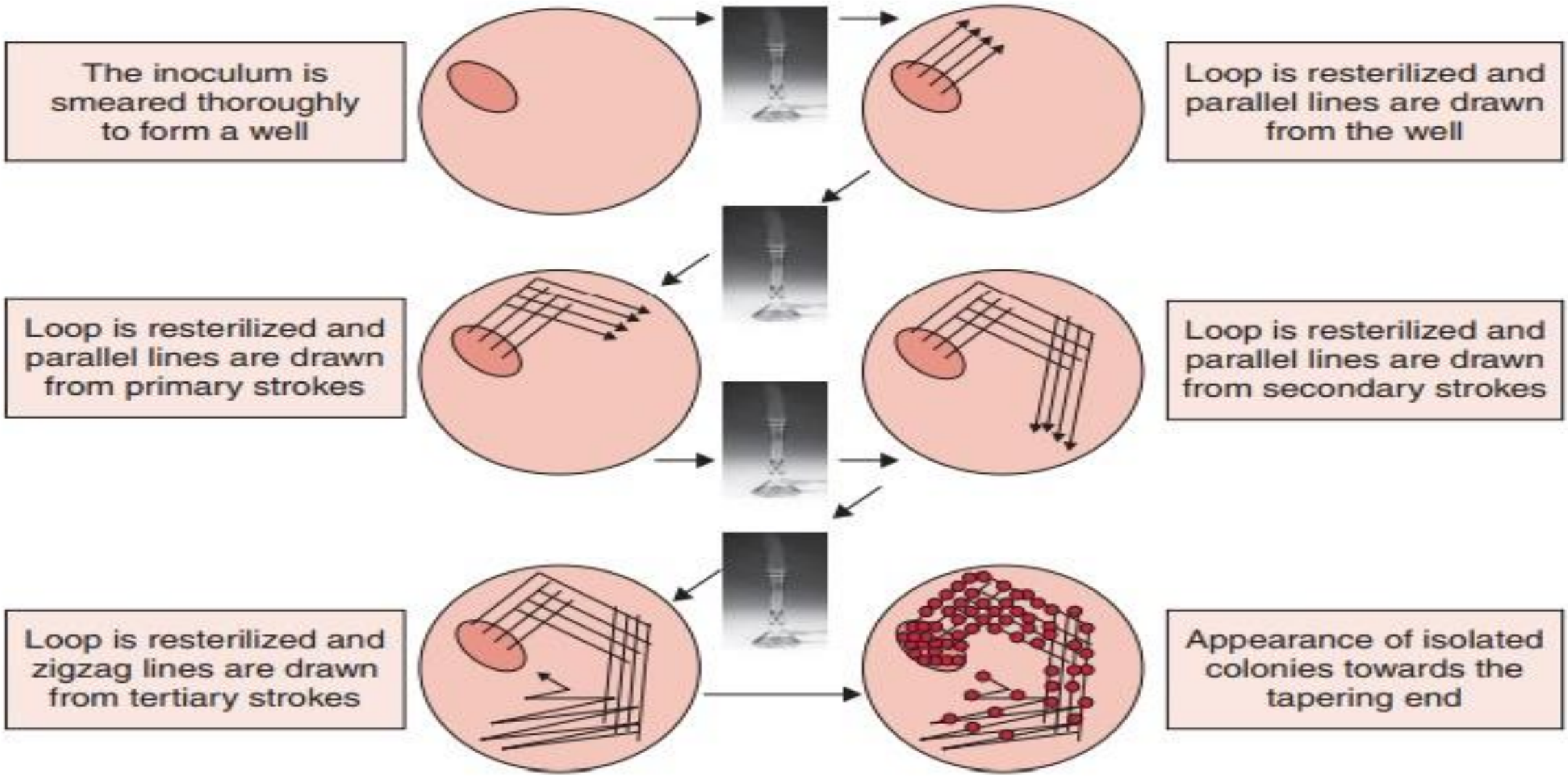


Streak culture

Aim to obtain pure culture i.e. an isolated growth of a single bacterium. Each bacterium divide repeatedly to give rise to a separate colony.

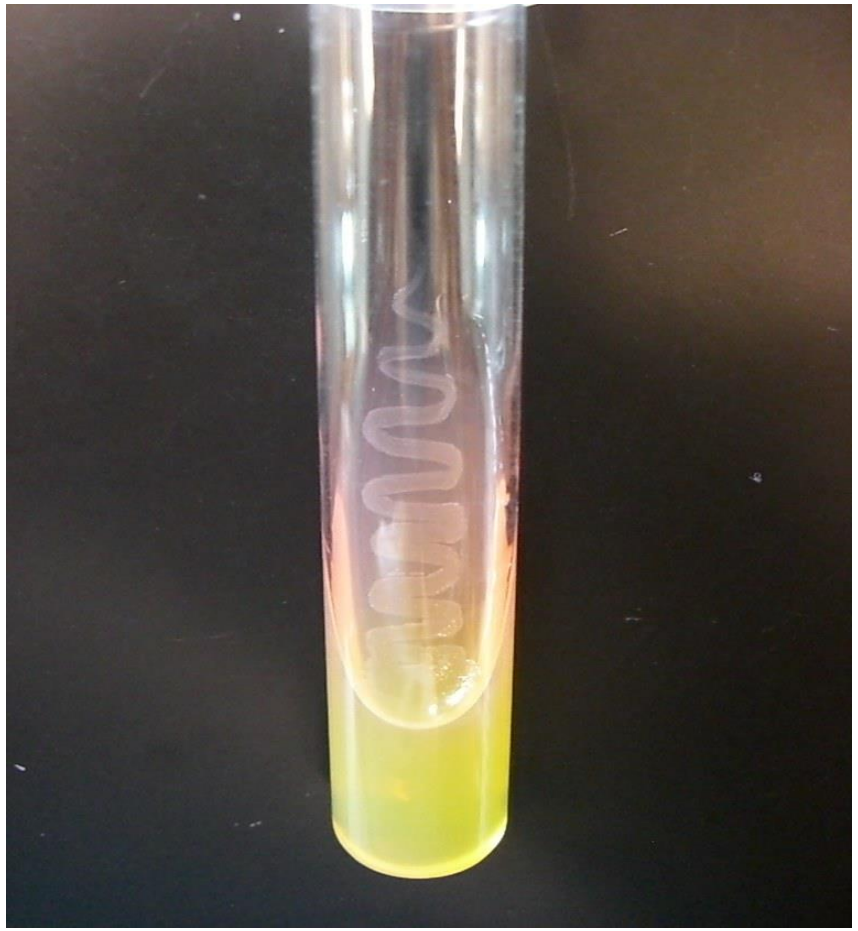


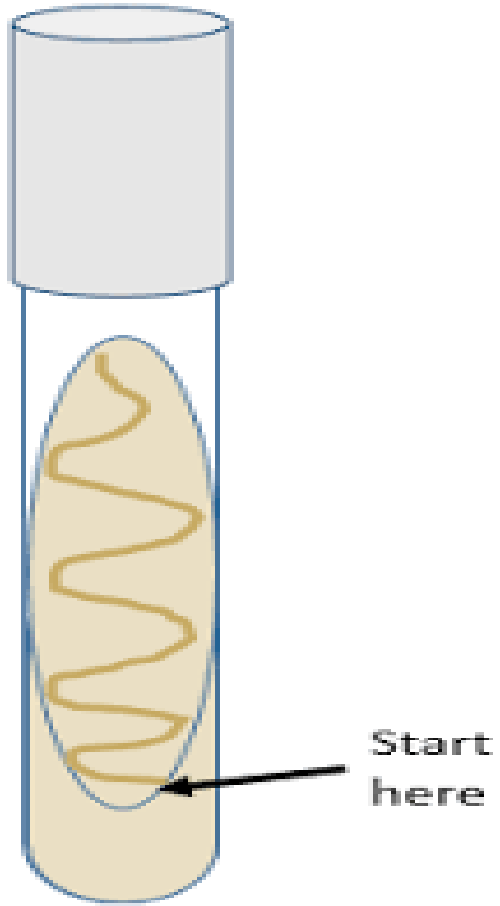
Streak culture



Slant culture

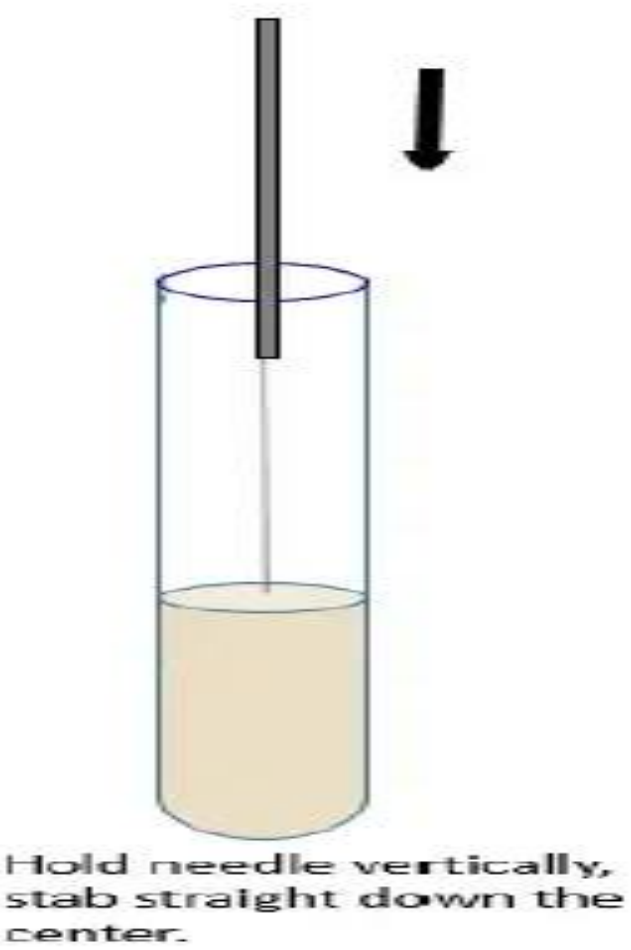
For performing biochemical tests
e.g. Triple sugar iron test.



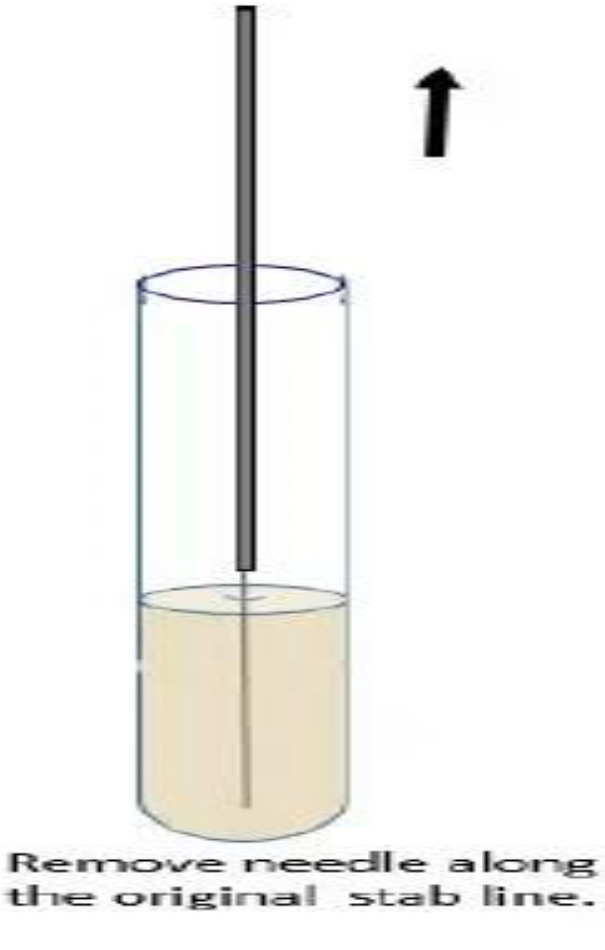


Stab culture

For motility test



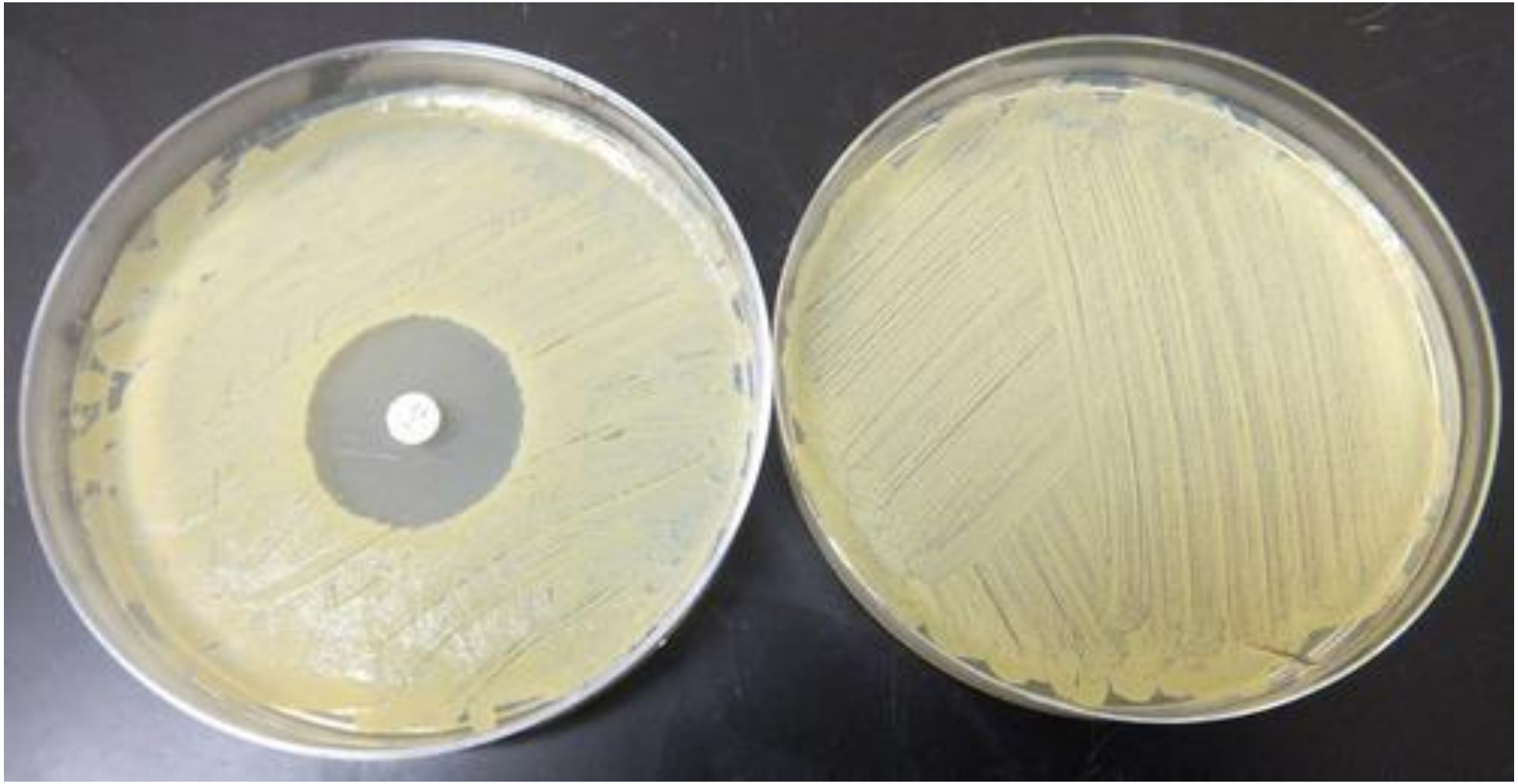
Hold needle vertically, stab straight down the center.



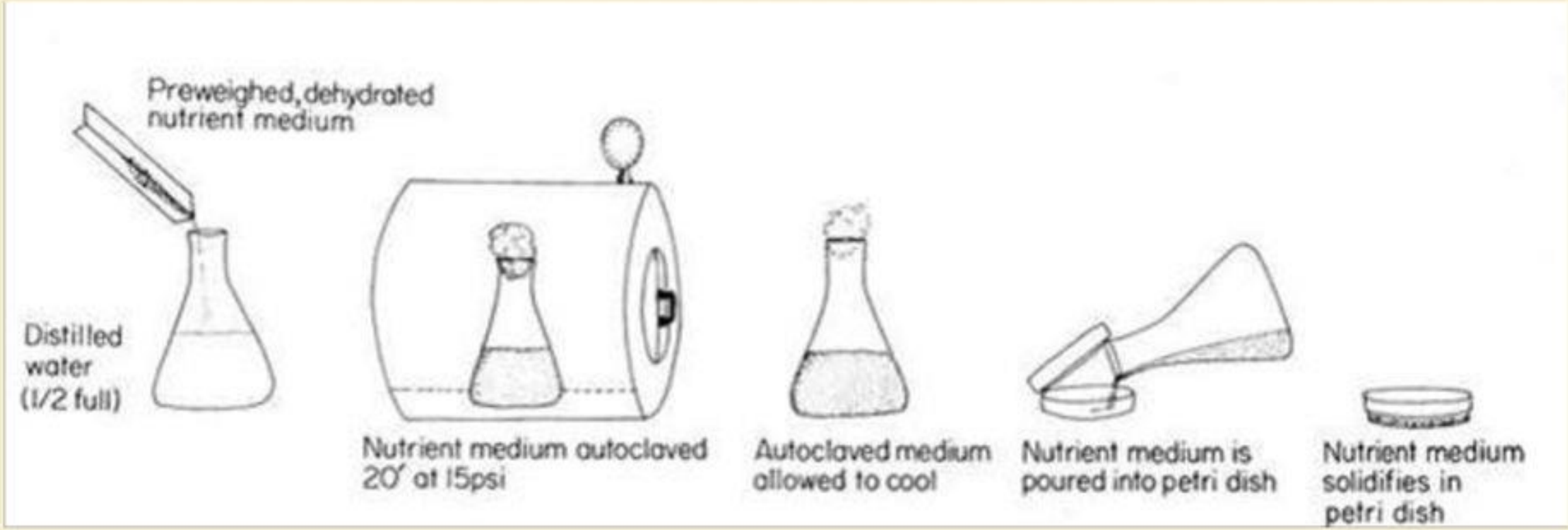
Remove needle along the original stab line.

Lawn culture

For antibiotic sensitivity (Disc diffusion)



Preparation of solid nutrient media in plate (Petri dishes)



Thank you