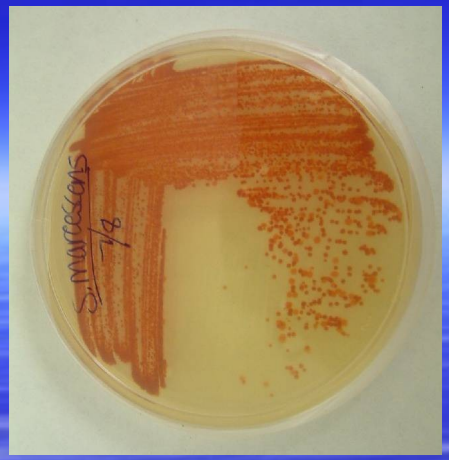
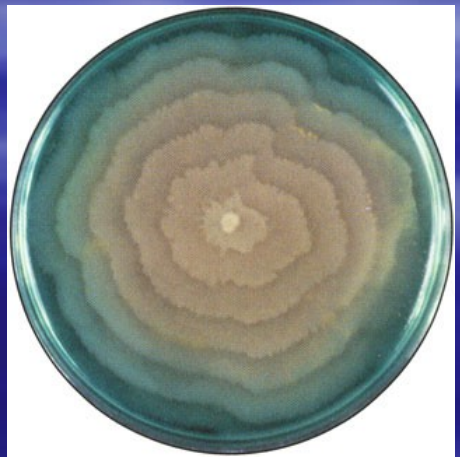


MOTILITY TEST & CULTURE MEDIA.

DR. F. R. BANGASH



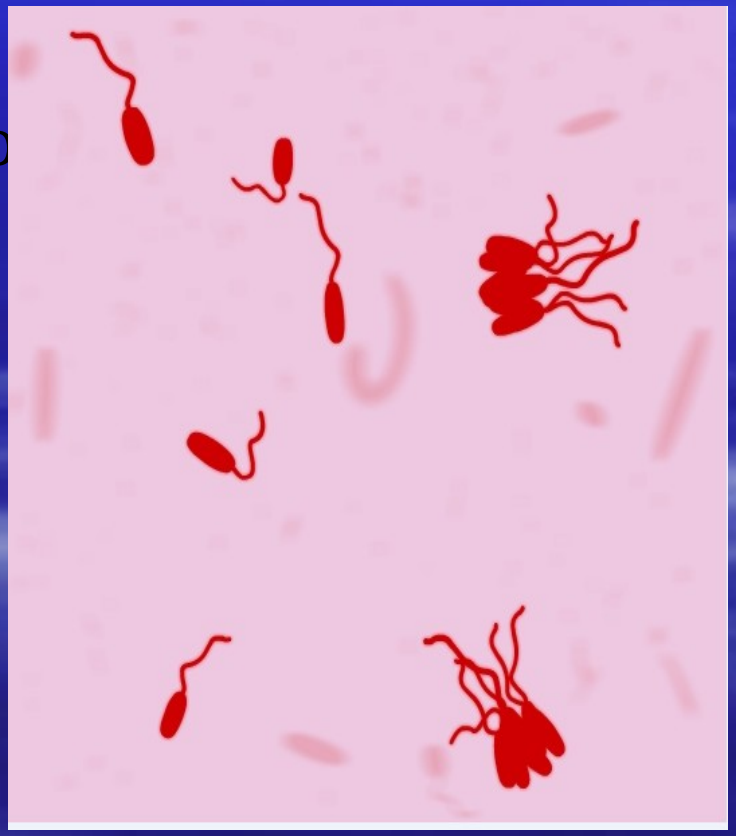
Site of flagella

Peritrichous

Monotrichous

(E.coli)

cho

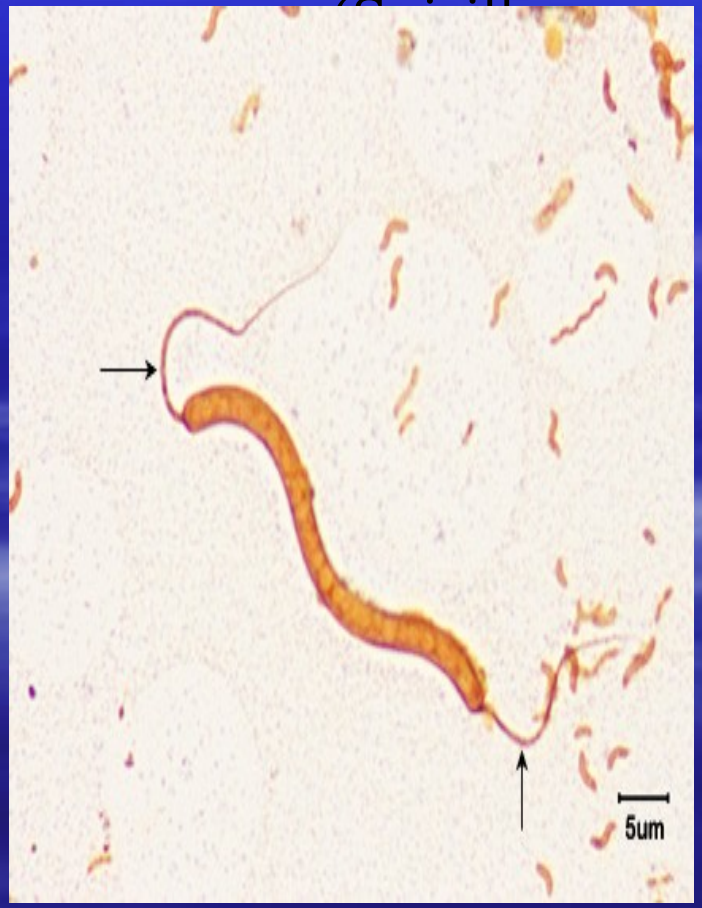


Site of flagella

Lophotrichous

Amphitrichous

(Pseudomonas)



Types of Motility.

1-True movement (depends on flagella).

2- False movement.

A- Brownian movement

- Vibratory movement

B- Drafting movement

- organisms streaming along a tide

Ways for detecting motility.

1- Flagella staining.

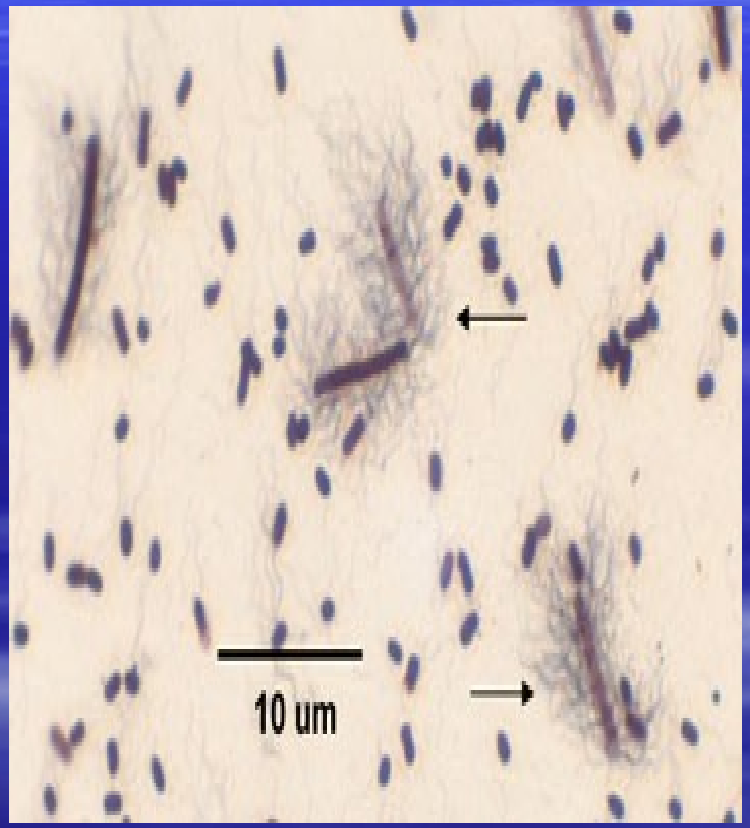
2- Motility test in semi-solid media.

3- Hanging Drop Technique.

1- Flagella staining method:

Rosanalin dye + ferric tannate

silver nitrate



2. Semi-Solid media Inoculation

The most commonly used test for motility in microbiology lab.

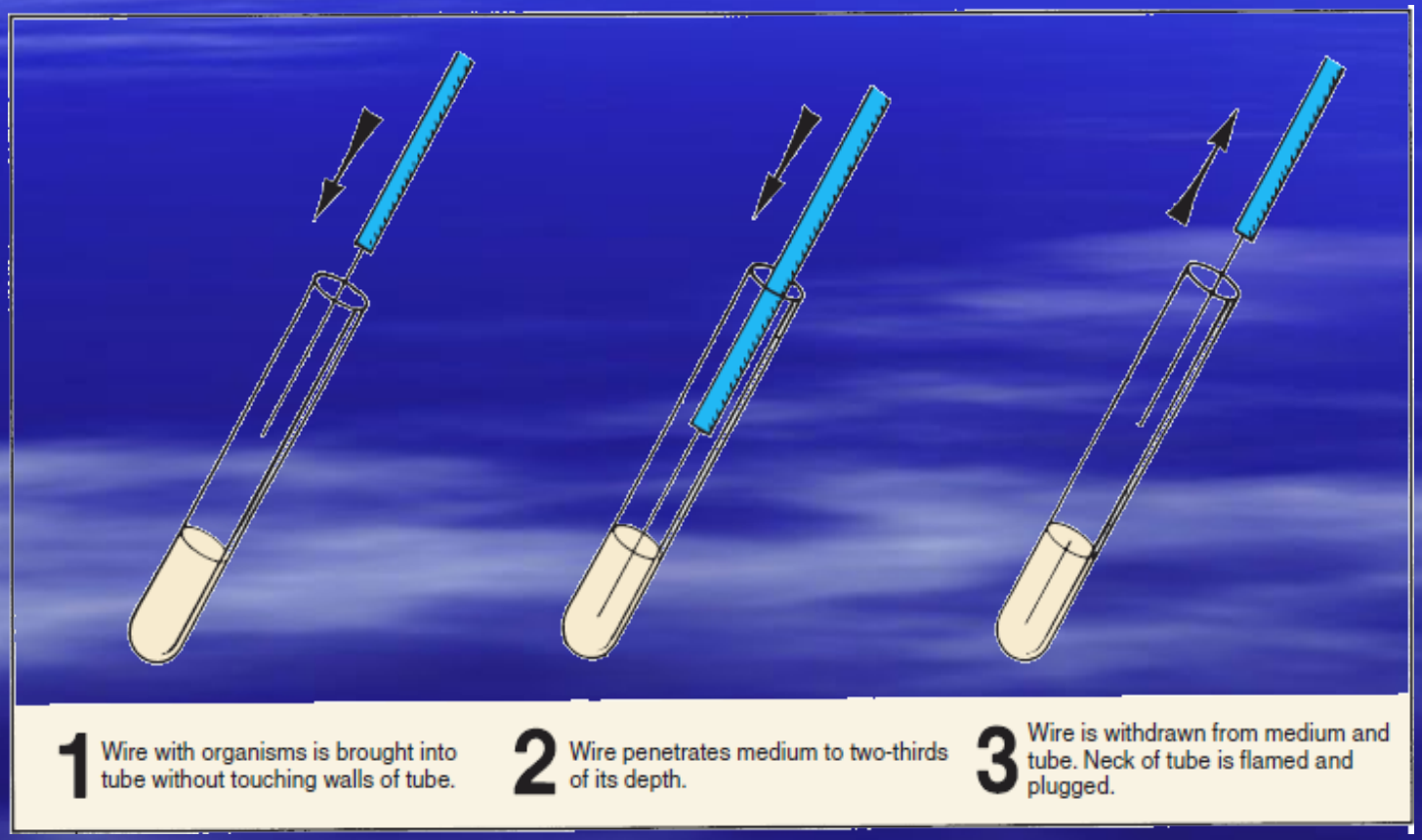
It depends on the ability of motile bacteria to move through semi-solid media.

Ordinary solid media contain 1.5-2.0% Agar

Semi solid media contain about 0.4% Agar

Procedure of Motility Test

- Using a sterile bacteriological needle, pick a colony of the test organism
- Stab quickly a tube of semi solid media. (avoid using bent needles).
- Incubate the semi solid media for 24 hours

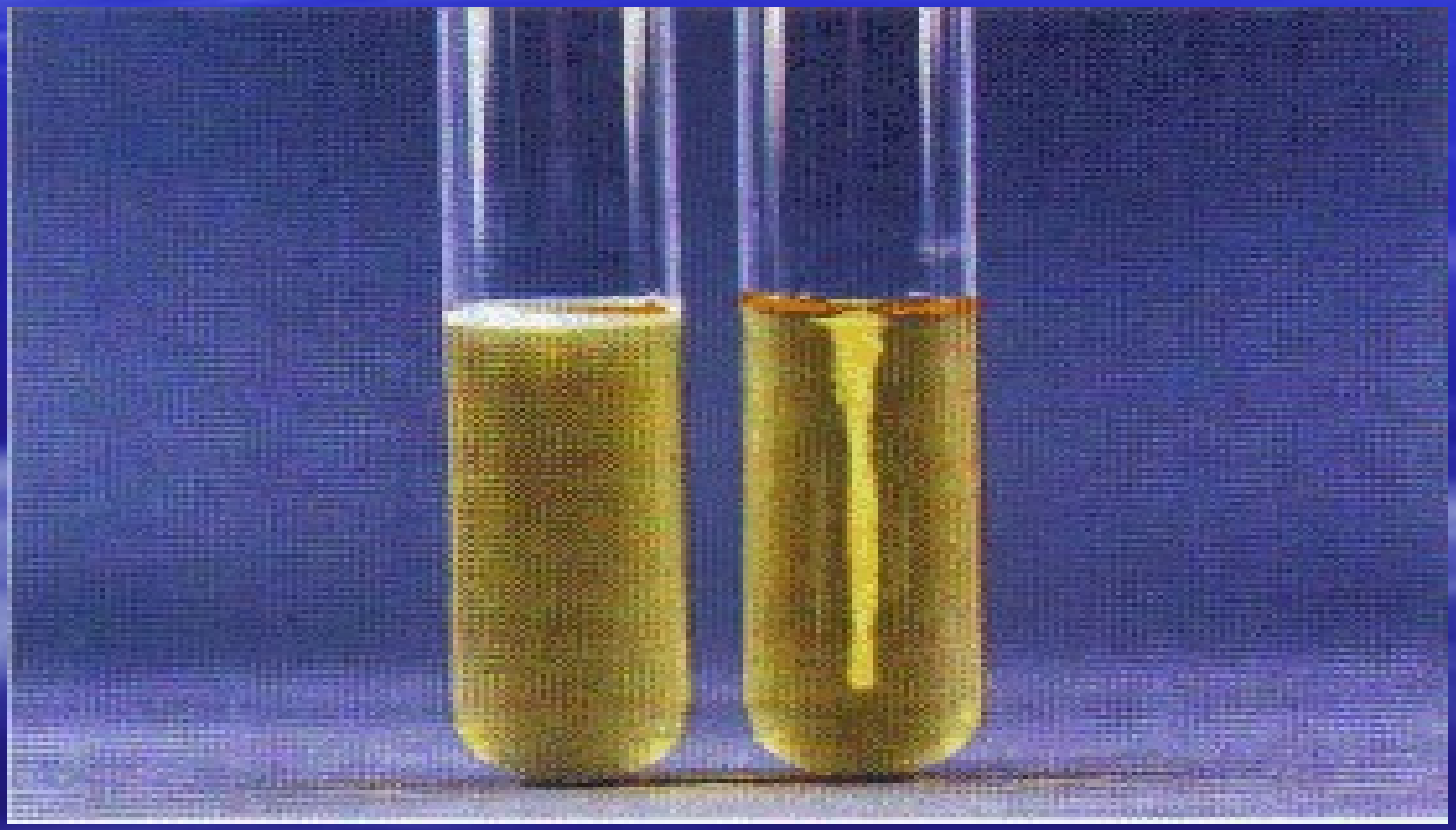


Reading Results:

If bacteria is motile, there will be growth going out away from the stab line, and test is positive.

If bacteria are not motile, there will only be growth along the stab line.

A colored indicator can be used to make the results easier to see.



Hanging Drop Method :

To observe bacteria in a wet mount and determine their motility.

Materials: 24-hour broth culture of *Proteus vulgaris*,

24-hour broth culture of *Staph. Epidermidis*,

2 hollow-ground slide,

Several cover glasses,

Wire inoculating loop,

Bunsen burner,

Petroleum jelly.

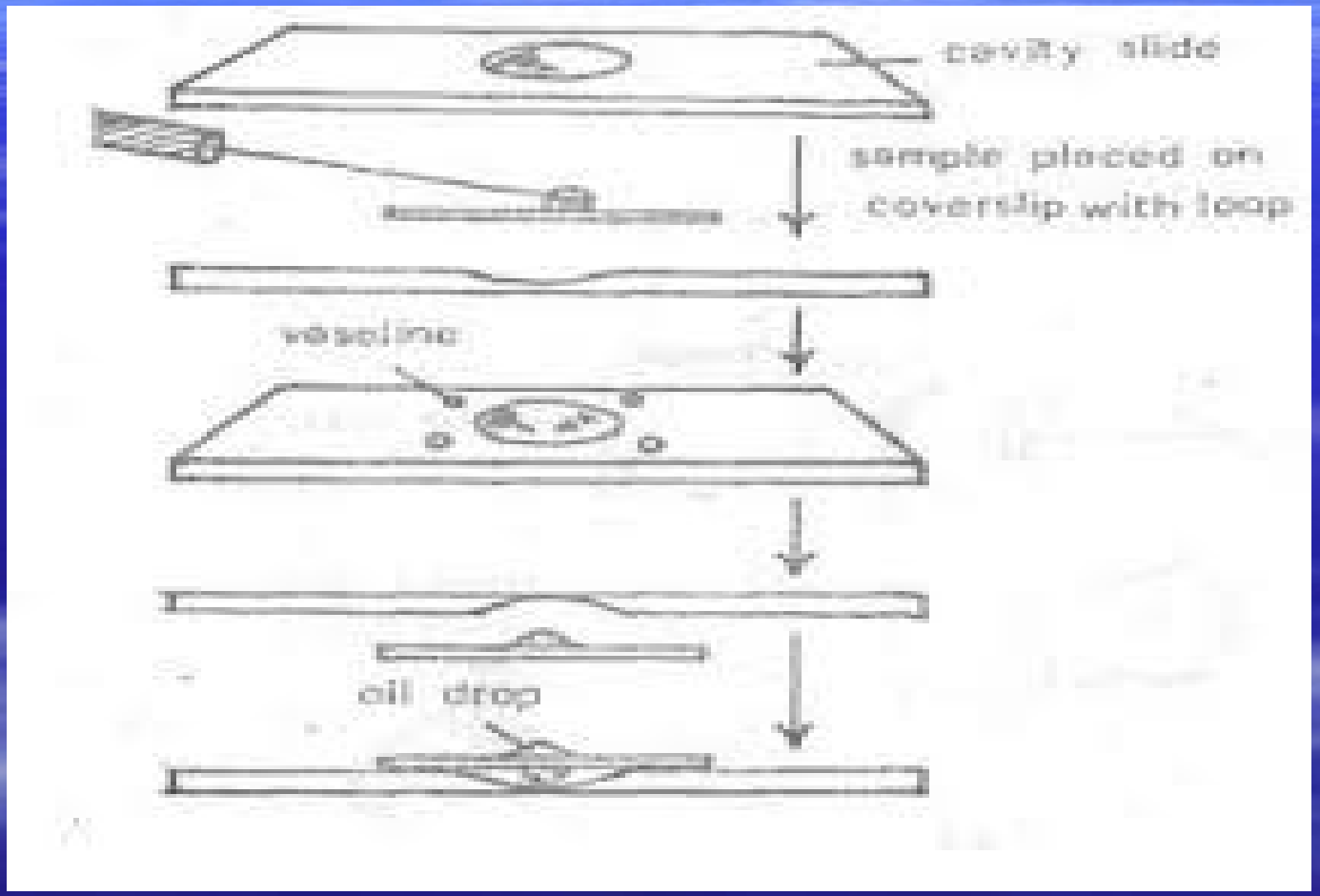
- **Procedures :**
  - 1-Take a clean cover glass or clean it thoroughly, making certain that it is free of grease (the drop to be placed on it will not hang from a greasy surface).
  - 2-Take one hollow-ground slide and clean it with a piece of dry tissue.
  - 3- Place a film of petroleum jelly around the rim of the well.
  - 4- Gently shake the broth culture of Proteus until it is evenly suspended. Using the wire inoculating loop, sterilize on the Bunsen flame, remove a loopful of culture.
  - 5- Close, and return the tube to the rack.

- **Procedures contd…**
- 6- Place the loopful of culture in the center of the cover glass (do not spread it around). Flame the loop and put it down.
- 7- Hold the hollow-ground slide inverted, well down, over the cover glass; then press it down lightly so that the petroleum jelly adheres to the four edges of the cover glass.

- 8- Now turn the slide over. You should have a sealed wet mount, the drop of culture hanging in the well.
- 9- Place the slide on the microscope stage, cover glass up.
- 10-Start examination with the low-power objective(40X) to find the focus and then with 100X.
- 11- It is helpful to focus first on one edge of the drop, which will appear as a dark line.

- 7- Continue your examination with the high-dry and oil immersion objectives (be very careful not to break the cover slip with the latter).
- 8- Make a hanging-drop preparation of the staphylococcus culture, following the same procedure described above.
- 9- Record your observation of the shape, cell groupings, and motility of the organisms.
- 10- Discard your slides in a container with disinfectant solution.

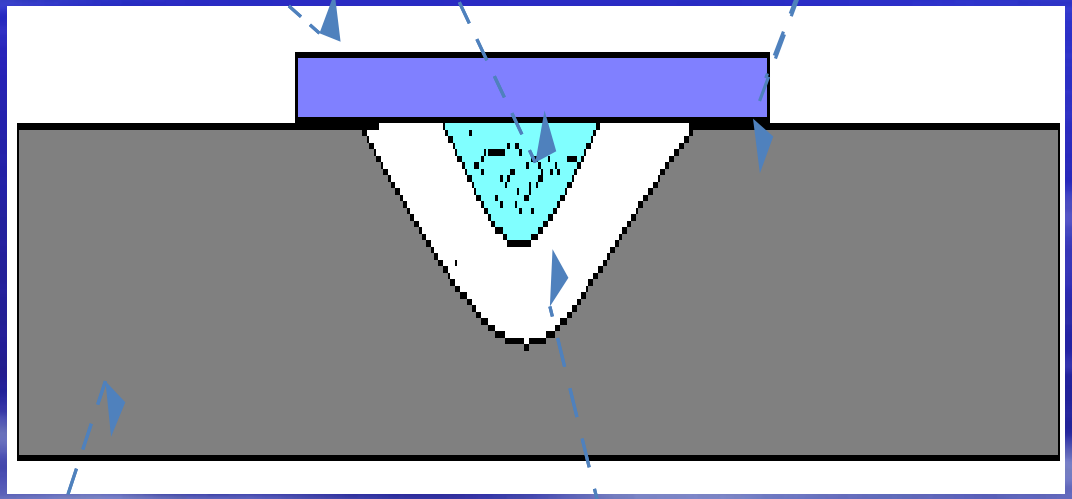
Hanging Drop Technique.



cover glass

Bacterial suspension

petroleum



Hollow ground slide

concave well

CULTURE MEDIA.

- Bacteria have to be grown (cultured) to be identified.
- By appropriate procedures they have to be grown separately (isolated) on culture media and obtained as pure for study.

History

- The original media used by Louis Pasteur – urine or meat broth
- Liquid medium – diffuse growth
- Solid medium – discrete colonies.

COLONY: Macroscopically visible collection of millions of bacteria originating from a single bacterial cell.

- Cooked cut potato by Robert Koch - Earliest solid medium.
- Gelatin – Not satisfactory as it liquefies at 24^{o}C.

AGAR:

- First described for use in microbiology in 1882 by a German microbiologist **Walther Hesse**, an assistant working in Robert Koch's laboratory.
- Used for preparing solid medium.
- Obtained from seaweeds.
- No nutritive value.
- Not affected by the growth of the bacteria.
- Melts at 98^{o}C & sets at 42^{o}C.
- 2% agar is employed in solid medium.

Types of Culture Media:

I. Based on their consistency:
   a) Solid medium,
   b) Liquid medium,
   c) Semi solid medium.

II. Based on the constituents/ ingredients:
   a) Simple medium,
   b) Complex medium,
   c) Synthetic or defined medium,
   d) Special media.

III.Based on Oxygen requirement:

- Aerobic media,
- Anaerobic media.

***SPECIAL MEDIA*:**

- Enriched media,
- Enrichment media,
- Selective media,
- Indicator media,
- Differential media,
- Sugar media,
- Transport media,
- Media for biochemical reactions.

Solid media – contains 2% agar.

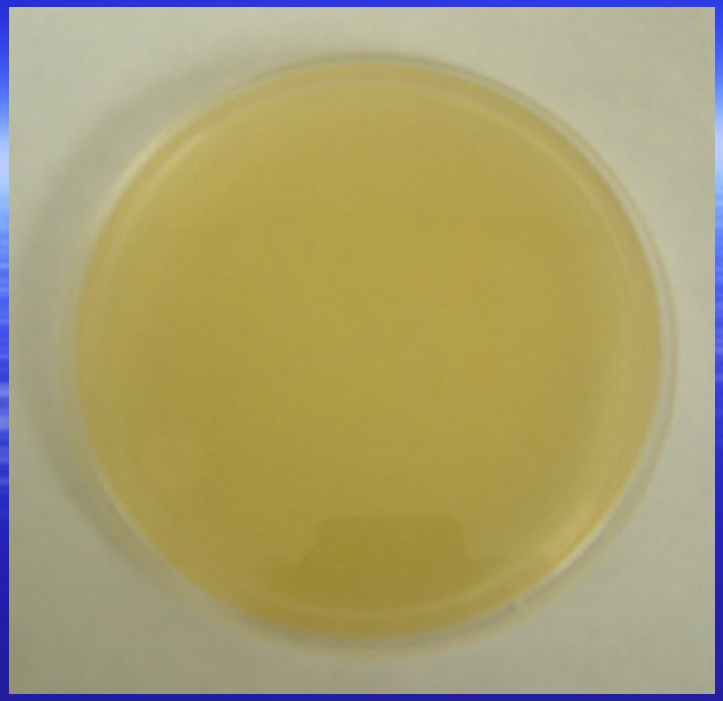
- Colony morphology, pigmentation & hemolysis can be appreciated.
- E.g. Nutrient agar, Blood agar.

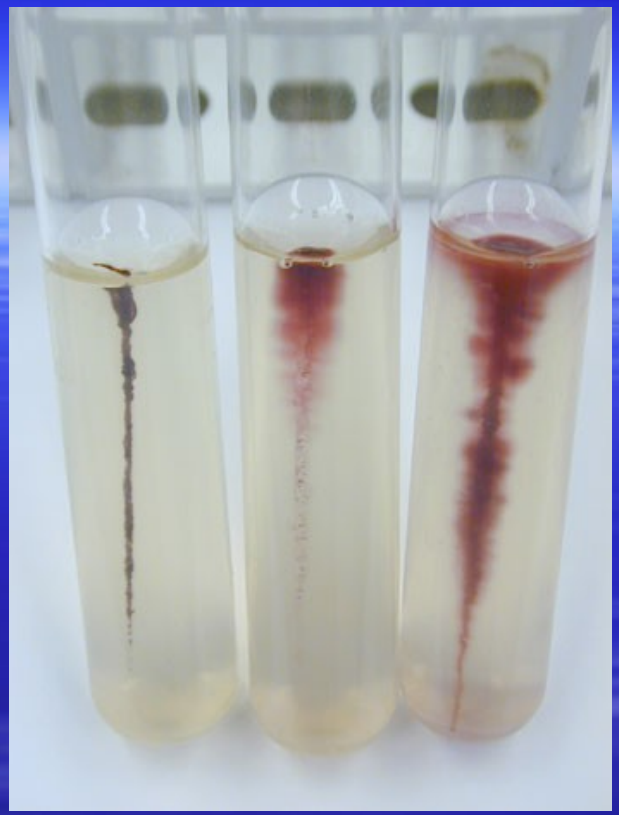
Liquid media – contains no agar.

- Can be used for inoculum preparation, Blood culture and for the isolation of pathogens from a mixture.
- Eg: Nutrient broth.

Semi solid medium – contains 0.5% agar.

- E.g. Motility medium.





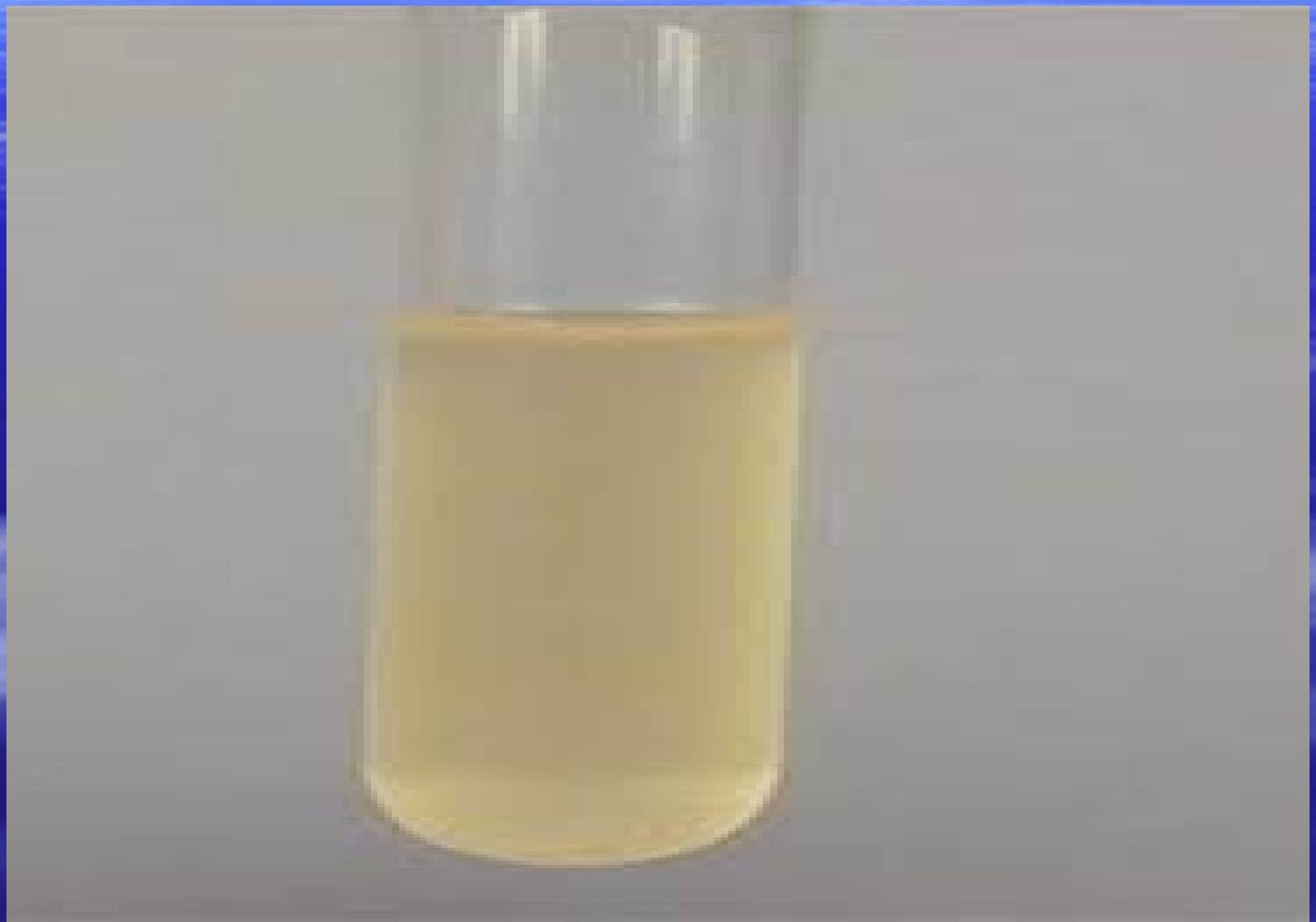
Important Media.

- Nutrient Broth,
- Nutrient Agar,
- Blood Agar,
- Chocolate Agar,
- MacConkey Agar,
- Lowenstein Johnson Medium(L.J. Medium),
- Sugar Medium,
- Cystine Lactose Electrolyte Deficient Medium (Cled Medium).

Nutrient Broth.

- Consists of peptone,NaCl, meat extract & D. water.
- Used for cultivating non fastigious bacteria & in preparation of enriched media like blood agar.

Nutrient Broth.



Nutrient agar.

- Nutrient broth + 2% agar.
- Colorless to light yellow.
- Basic culture medium containing basic essentials required for growth of bacteria.
- Used for culture and growth of bacteria.
- Also used to observe sensitivity of bacteria.

Nutrient agar.

Blood Agar.

- Nutrient agar + 5-10% animal blood.
- Red in color.
- Used for most of pathogenic bacteria except a few one, when specimen is faeces:
  - To differentiate between Strptococci on the basis of haemolysis.
  - Used to grow +ve organism e.g. staphylococci.
  - Can be made selective by adding chemicals, dyes or antibiotics.

Blood Agar.

Chocolate Agar.

- It becomes chocolate agar when blood agar is heated to 80 degrees celcius for 10 minutes.
- Color is chocolate brown.
- Used for N. Meningitis, H. Influenzae, Strpt. Pneumoniae, Staph. Aureus & mixed infections.

Chocolate Agar.

MacConkey Agar.

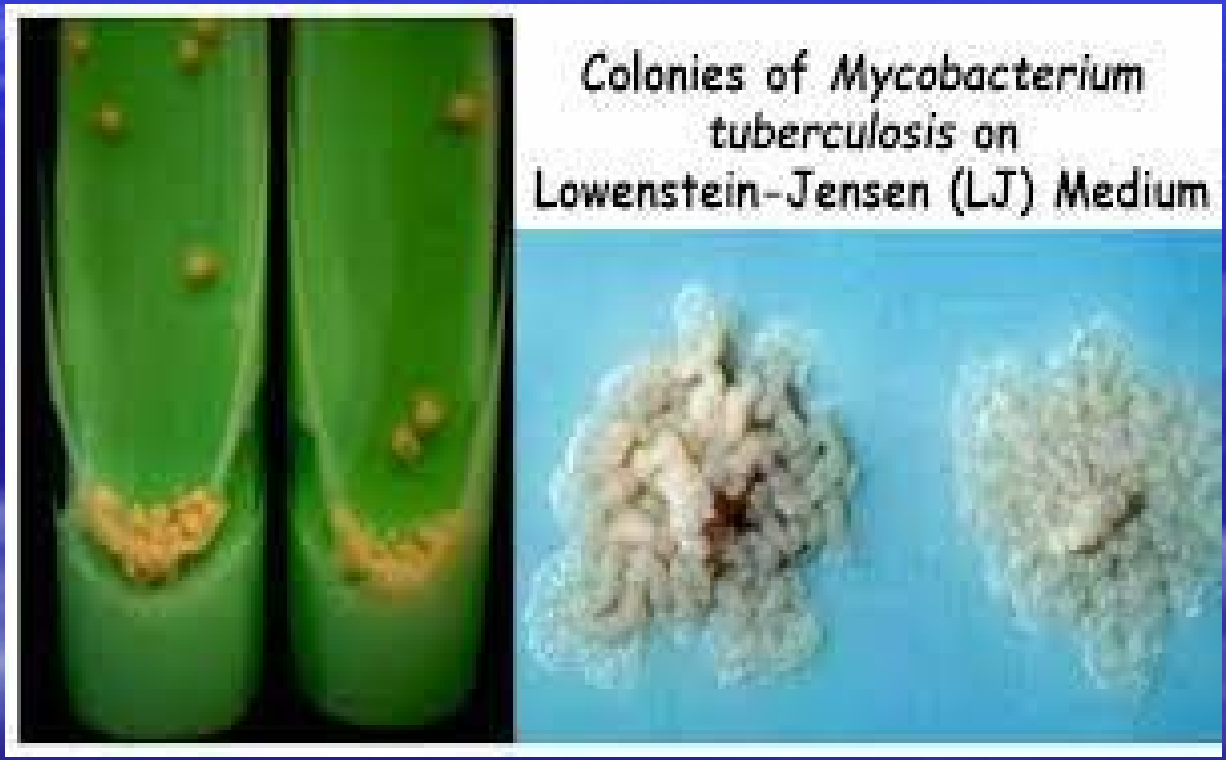
- Contains peptone, bile salt, lactose, neutral red(indicator), agar & distilled water.
- Color is light pink.
- Uses are:
  - Selective & differential medium.
  - To differentiate lactose fermentors from non lactose fermentors.
  - Inhibitory to strept. Pyogenes,Strept. Pneumoniae, Viridans group of Strptococci & Pasteurella.

MacConkey Agar.

Lowenstein Johnson(L.J.) Medium.

- Consists of asparagines, glycerol, malachite green, whole egg which give solid consistency to the medium & mineral salts.
- Selective medium present in screw capped bottle.
- Light green in color.
- Used for growth of Mycobacteria Tubercolosis.

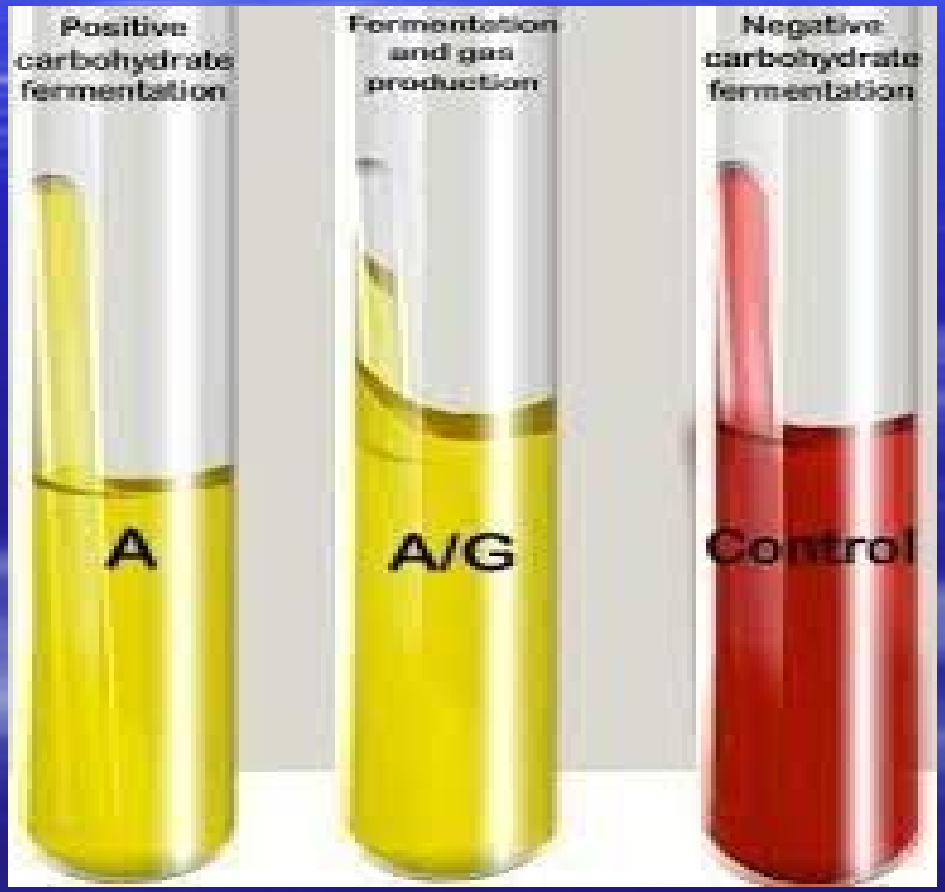
Lowenstein Johnson(L.J.) Medium.



Sugar Media.

- Sugar+ peptone water.
- Different color for different type of sugar:
  - Glucose→Green,
  - Lactose→Red,
  - Sucrose→Blue,
  - Mennite→Mauve,
  - Maltose→Blue,
  - Dulicite→Pink.
- Used for fermentation reaction i.e. production of acid or gas is noted.

Sugar Media.


Positive carbohydrate fermentation
A
Fermentation and gas production
A/G
Negative carbohydrate fermentation
Control

Cystine Lactose Electrolyte Deficient(CLED) Medium.

- Consists of cystine & lactose.
- Transparent & light green in color.
- Non inhibitory & differential medium mainly used for wine culture.

Cystine Lactose Electrolyte Deficient(CLED) Medium.