Isolation of bacteria

Purpose:

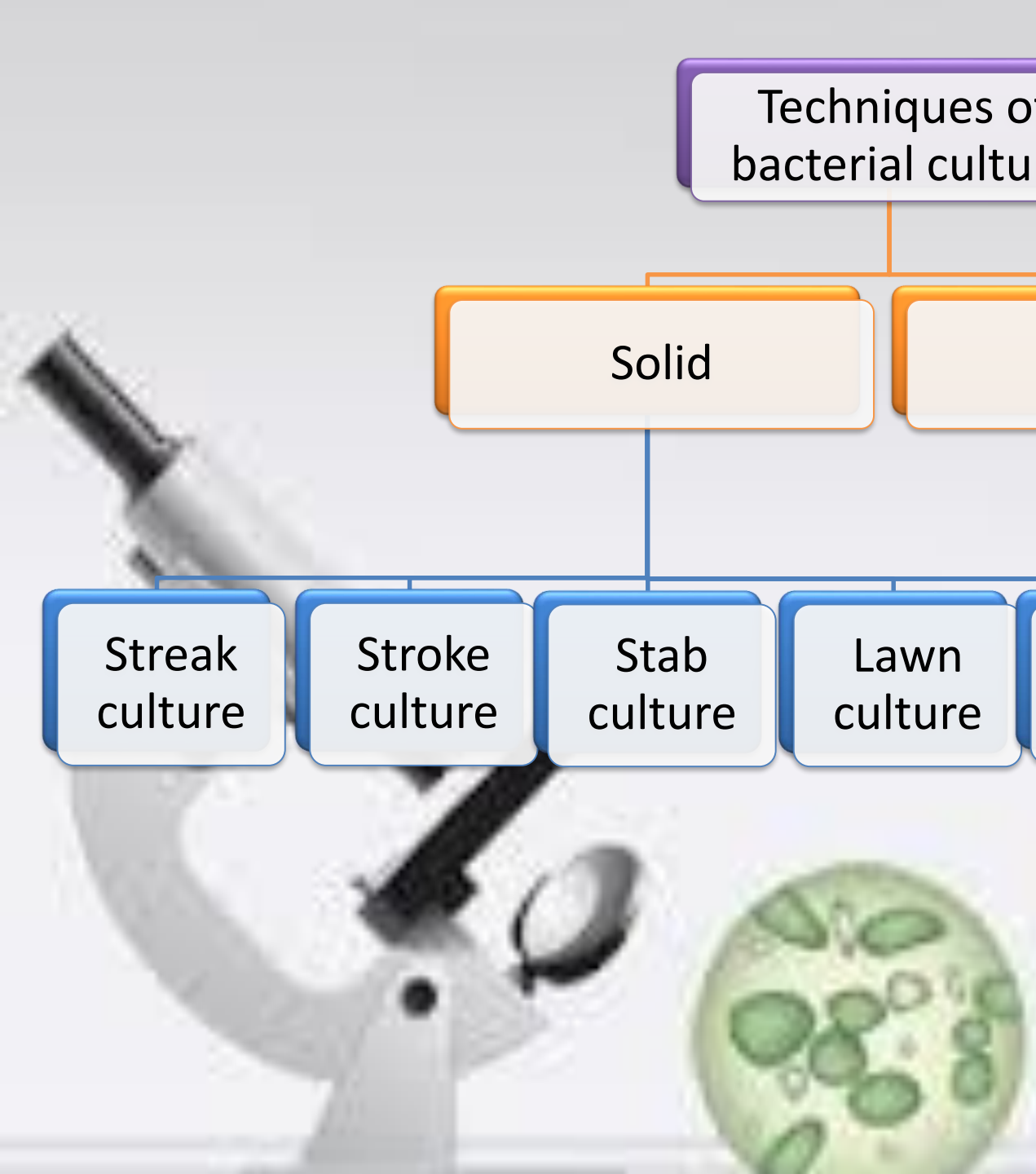
- To isolate bacteria in pure culture.
- To demonstrate their properties.
- To obtain sufficient growth for antigen preparation.
- For typing of bacteria.
- To determine antibiotic sensitivity.

Method:

- Bacterial culture- By growing the organism on artificial culture media at suitable temperature, pH and redox potential.
- By use of laboratory animals.

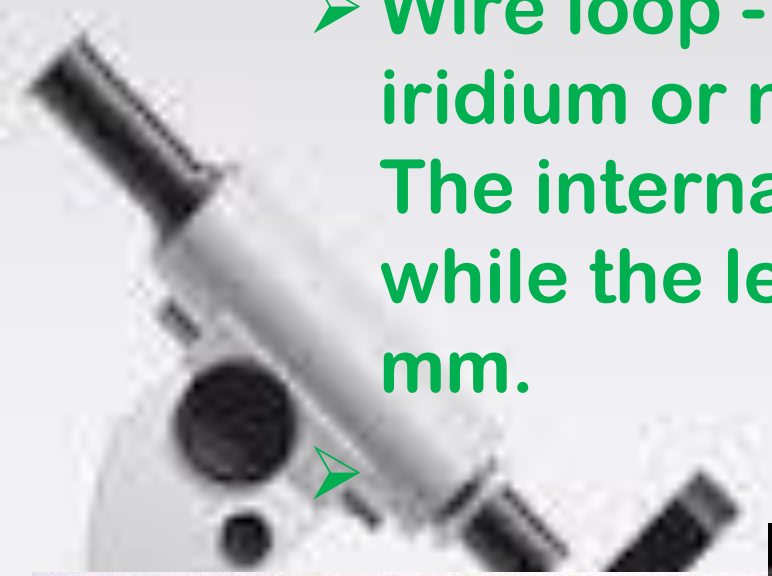
Techniques of bacterial culture

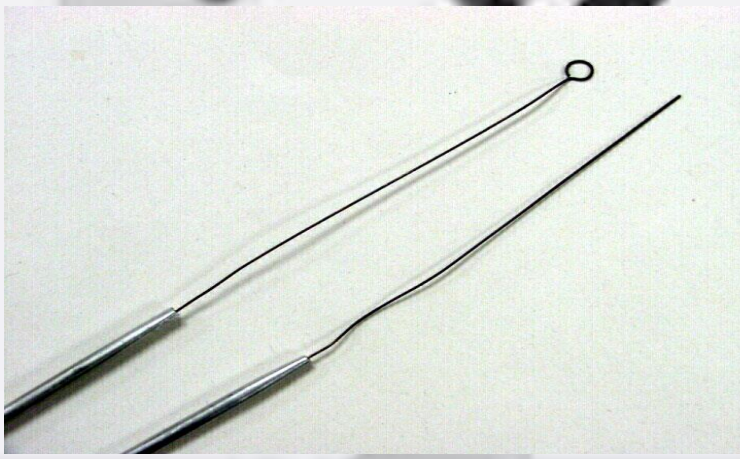
- Solid
  - Streak culture
  - Stroke culture
  - Stab culture
  - Lawn culture
  - Pour plate
  - Sweep culture
- Liquid

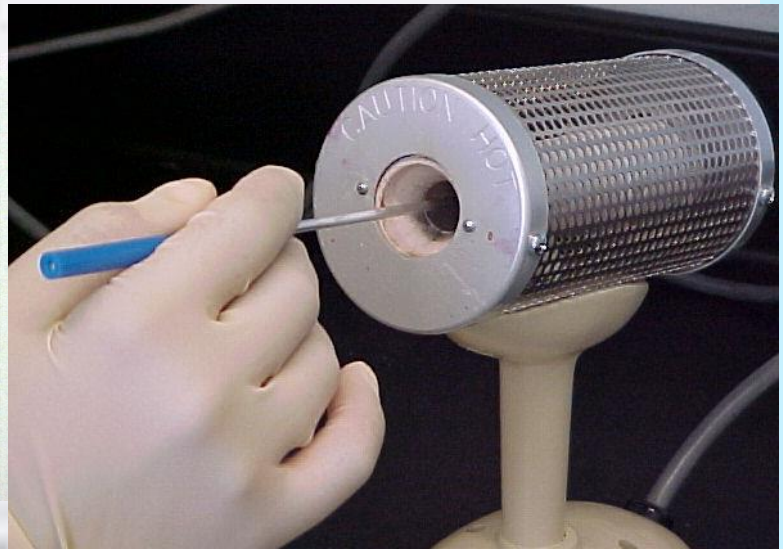


Instruments used to seed culture media

- Burner - Pilot flame is used.
- Straight wire.
- Wire loop - Made up of either platinum - iridium or nichrome or stainless steel. The internal diameter of loop is 2-4 mm while the length of the wire should be 50 mm.
- Sterile pipettes.

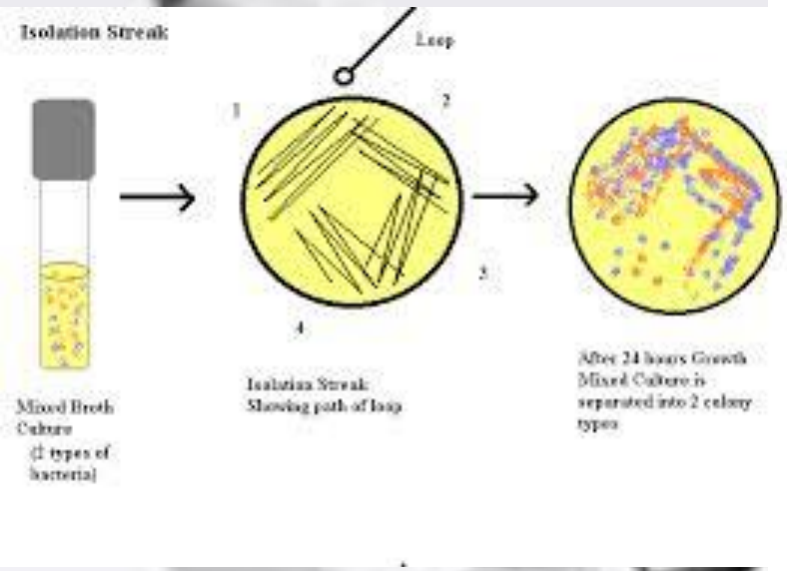


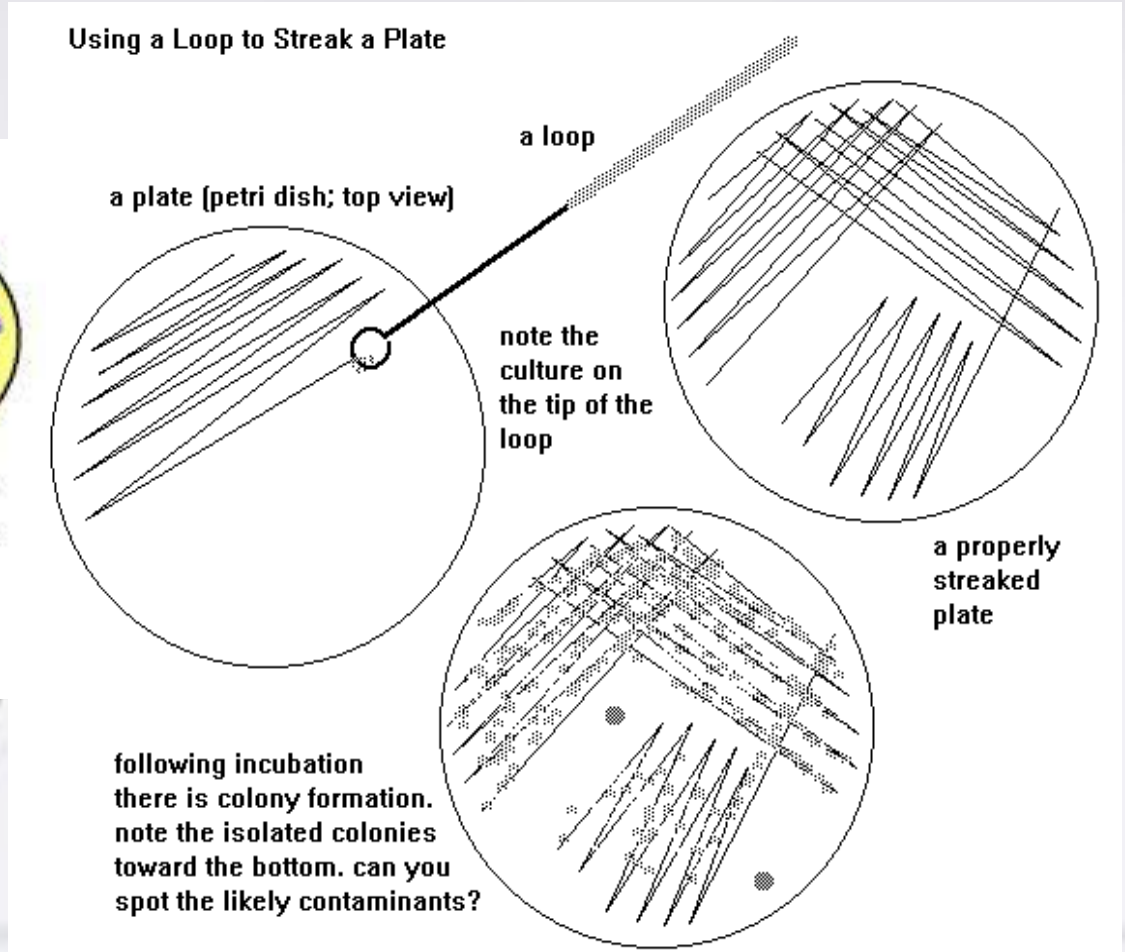




Streak culture:

It is the method routinely employed for the isolation of bacteria in pure culture from clinical specimens.





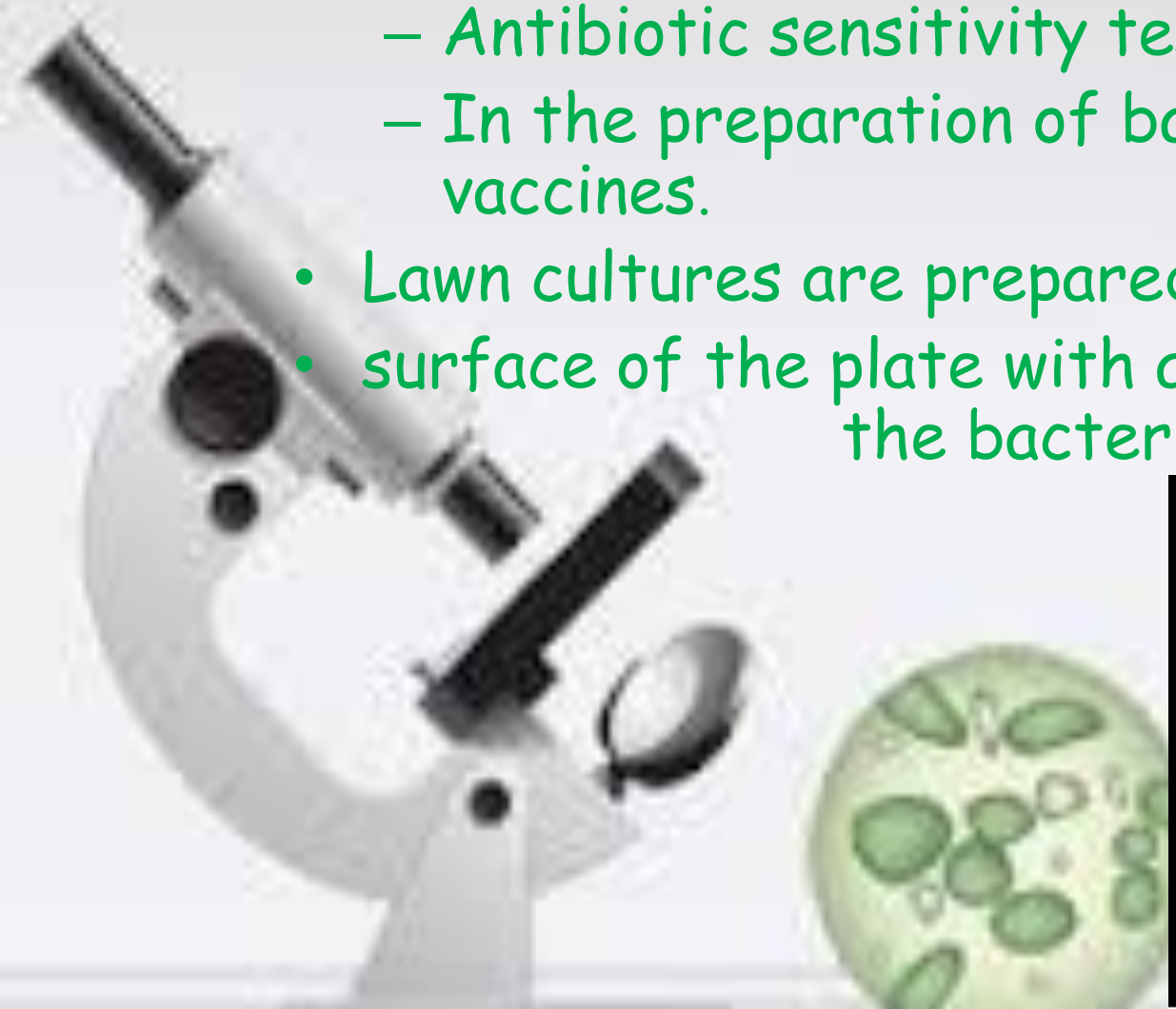
Stroke culture:

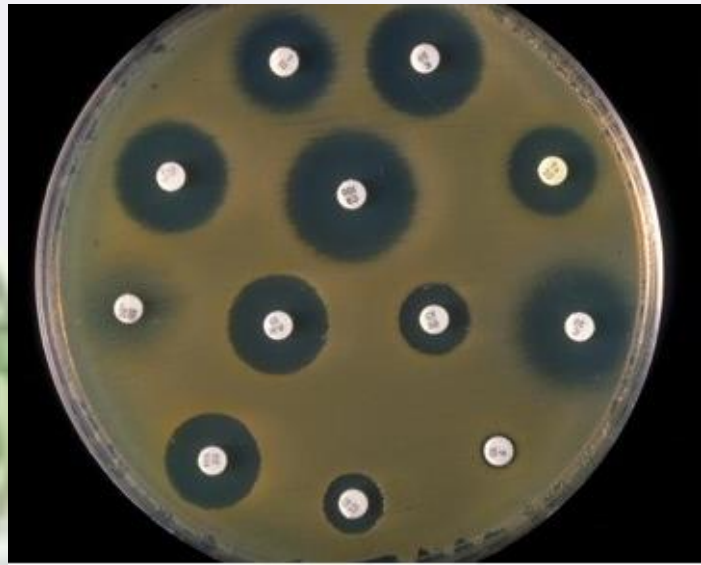
Uses: For preservation of bacterial cultures.

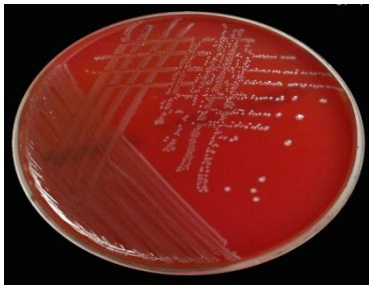
- For obtaining pure growth.
- For biochemical reaction

LAWN OR CARPET CULTURE

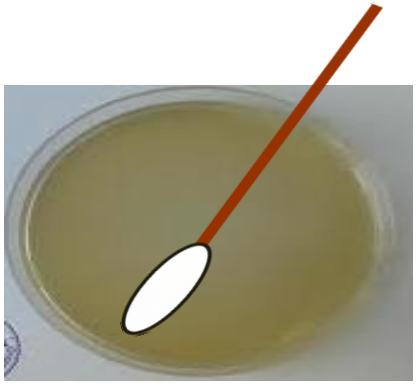
- Provides a uniform surface growth of the bacterium.
- Uses-For bacteriophage typing.
  - Antibiotic sensitivity testing.
  - In the preparation of bacterial antigens and vaccines.
- Lawn cultures are prepared by flooding the
- surface of the plate with a liquid suspension of the bacterium.





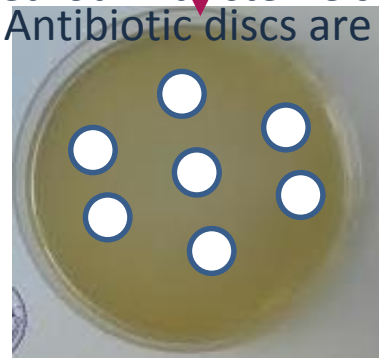


Suspension of organism is prepared in peptone water by similar colonies from agar plate.

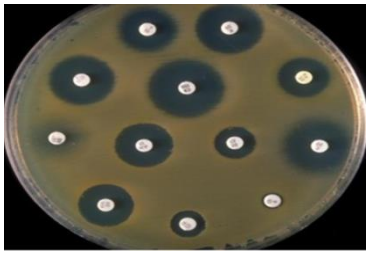


❑Suspension of organism is flooded on dried plate of agar by lawn culture method with sterile swab.

Antibiotic discs are kept



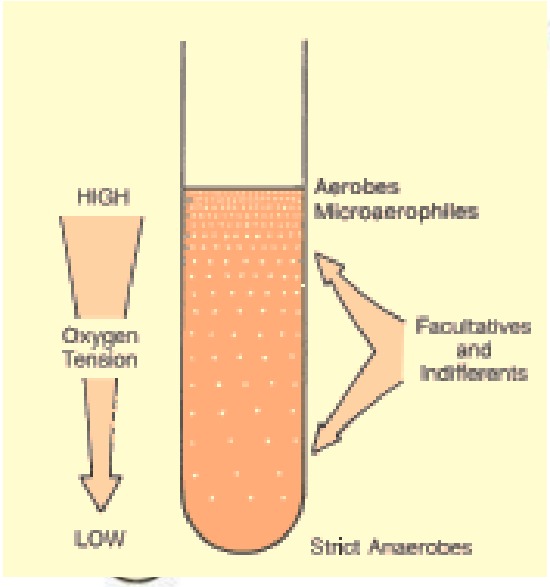
Plates are incubated for 18-24 hours

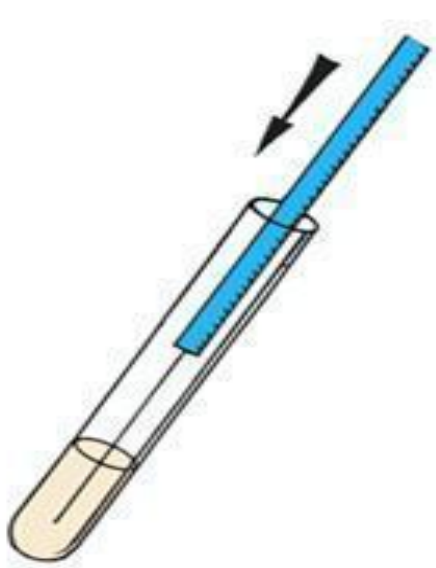


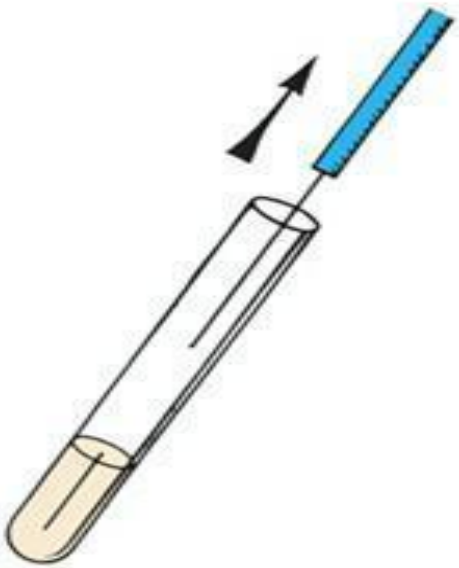
❑Zone of inhibitions around the disc is to be observed, after incubation period.

STAB CULTURE

- Prepared by puncturing a suitable medium – gelatin or glucose agar with a long, straight, charged wire.
- Uses-Demonstration of gelatin liquefaction.
  - Oxygen requirements of the bacterium under study.
  - Maintenance of stoke cultures.







1 Wire with organisms is brought into tube without touching walls of tube.

2 Wire penetrates medium to two-thirds of its depth.

3 Wire is withdrawn from medium and tube. Neck of tube is flamed and plugged.

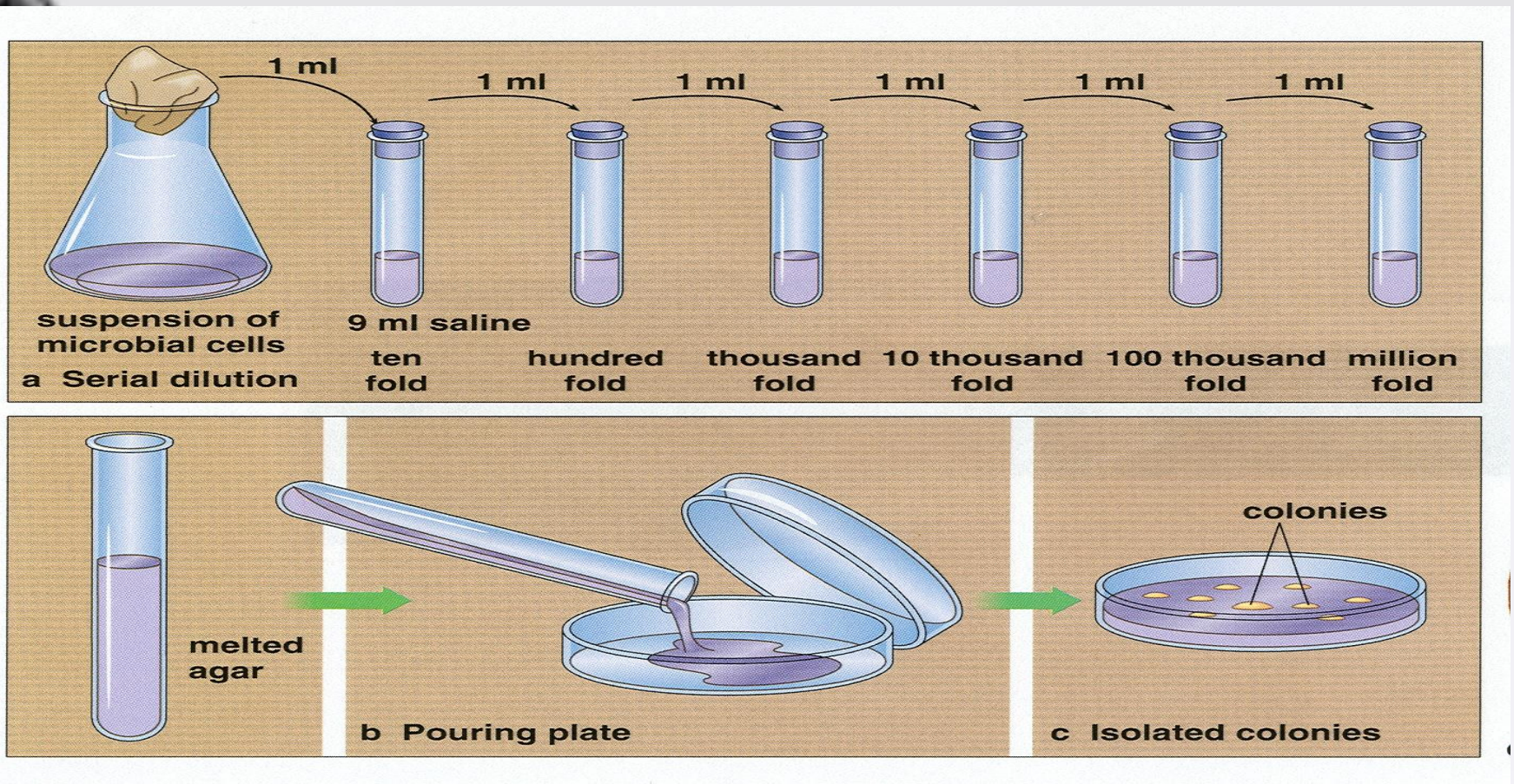
Pour plate culture

- The Inoculum to be tested is serially diluted.
- Known amount of each dilution is mixed with 15 ml of melted agar at 45-50°c and mixed well.
- The content of the tube are pored in a sterile petridish and allowed to set.
- After overnight incubation at 37°C , the colonies distributed throughout the depth of the media are counted.
- The number gives the viable bacterial count in a given suspension.

Pour plate culture :

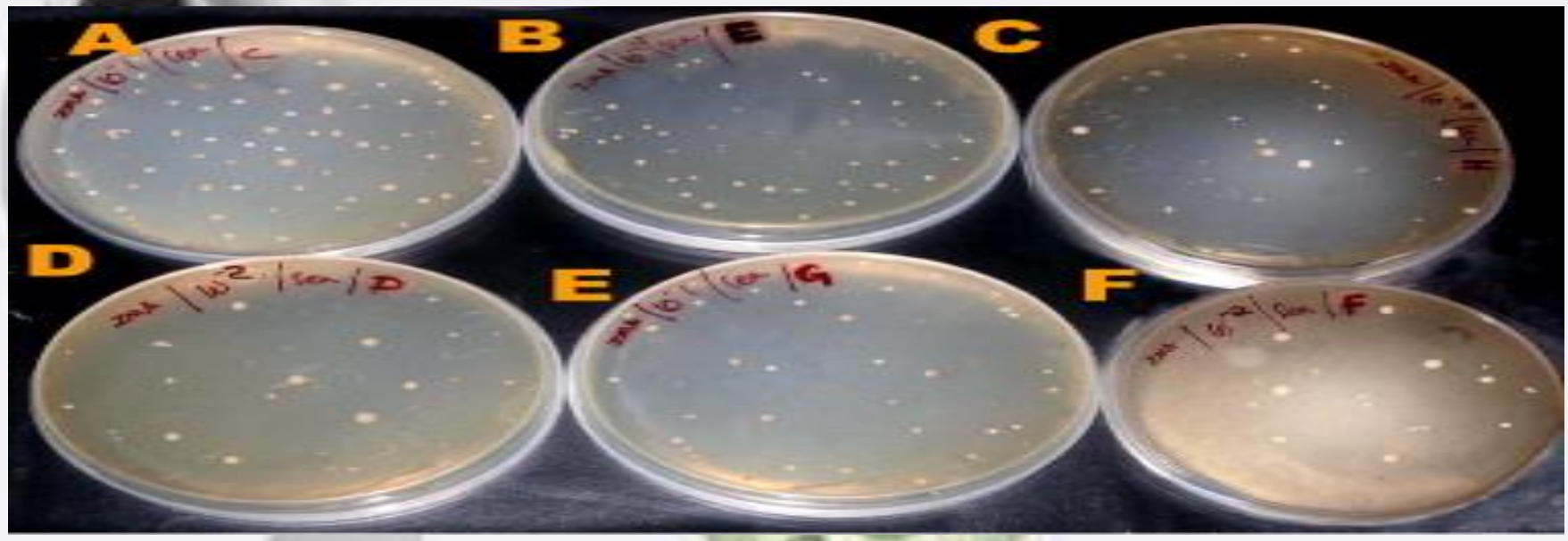
Uses :To quantify bacteria in urine culture

- **Analysis of water**
- **For assay of antibiotics and enzymes.**



Sweep plate :

The edges of the petridish containing culture media are rubbed over the fabric with the medium facing it. The dust particles stirred up from the cloth settle on the culture medium and colonies develop on incubation .They can be counted.



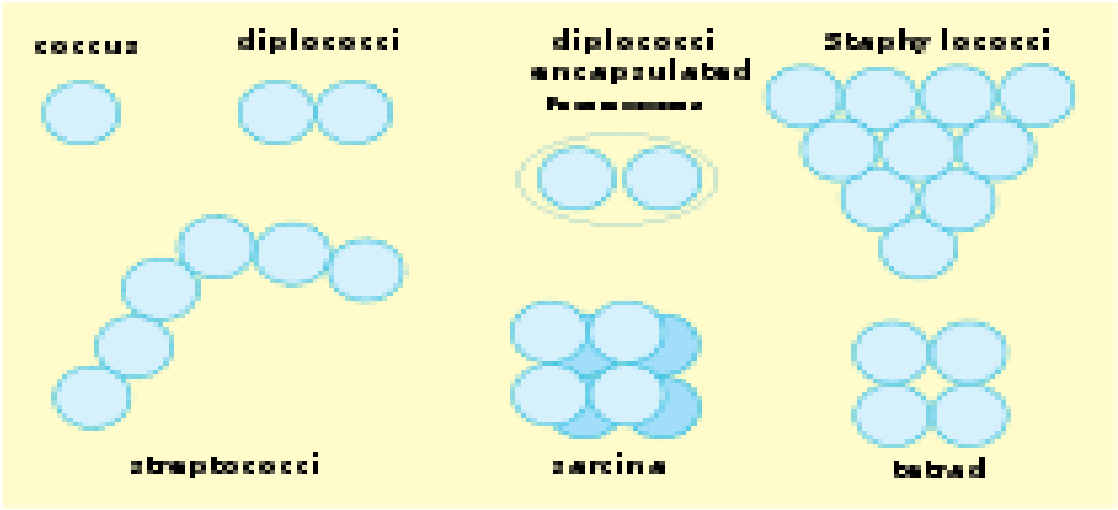
Identification of bacteria is done by studying the following characteristics:

- Morphological characteristics
- Cultural characteristics
- Biochemical reactions
- Agglutination by antisera
- Typing
- Animal inoculation

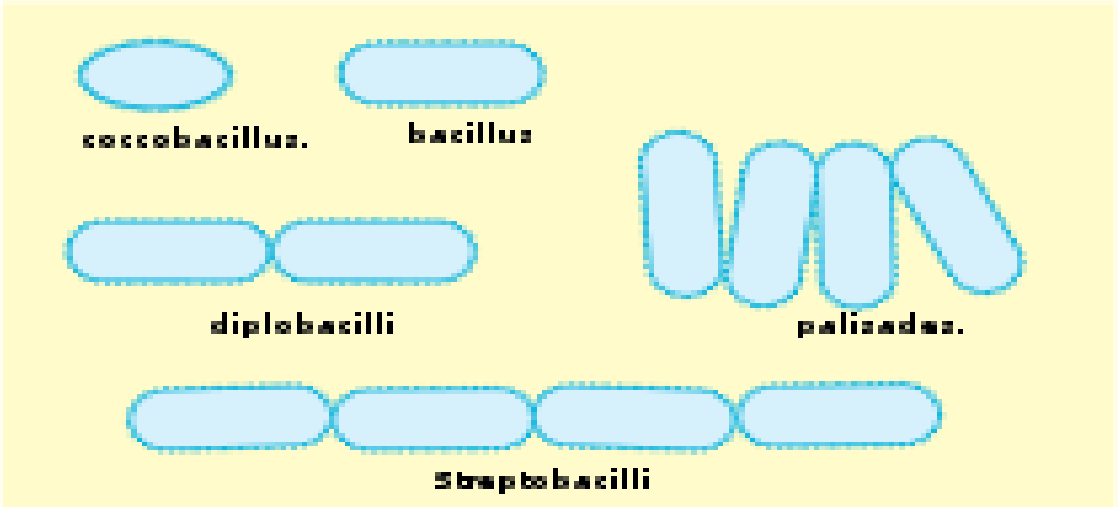
Morphology:

- Size
- Shape
- Arrangement
- Presence or absence of motility
- Presence or absence of spores & capsule
- Staining reaction

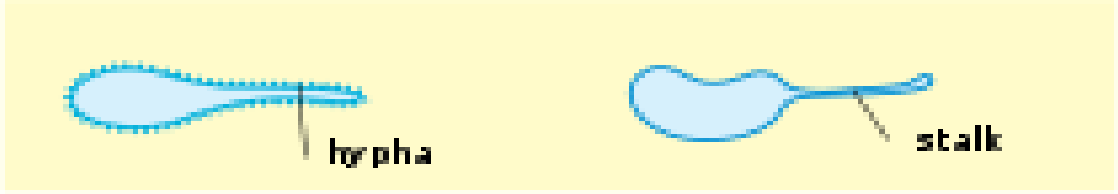
Cocci



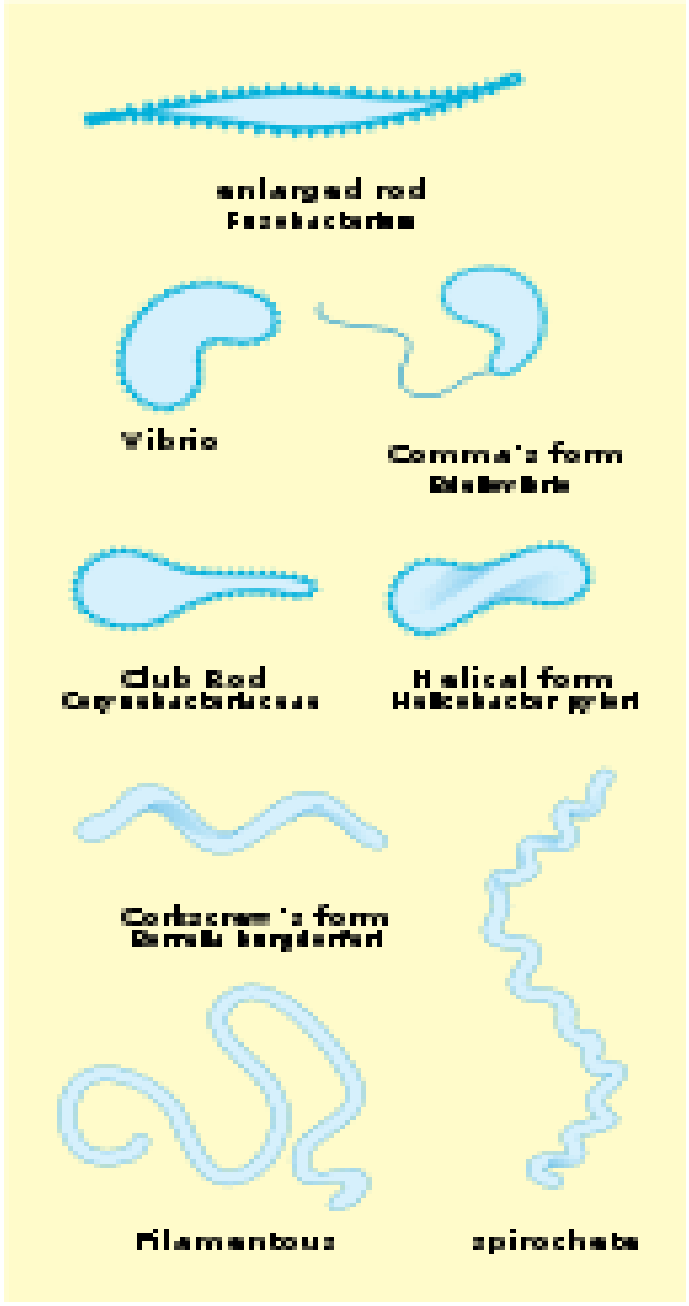
Bacilli



Budding and appendaged bacteria



Others



Colony- A bacterial population derived from one bacterial cell. The cells within the colony have identical, genus, species, genetic and phenotypic characteristics.

Colonies

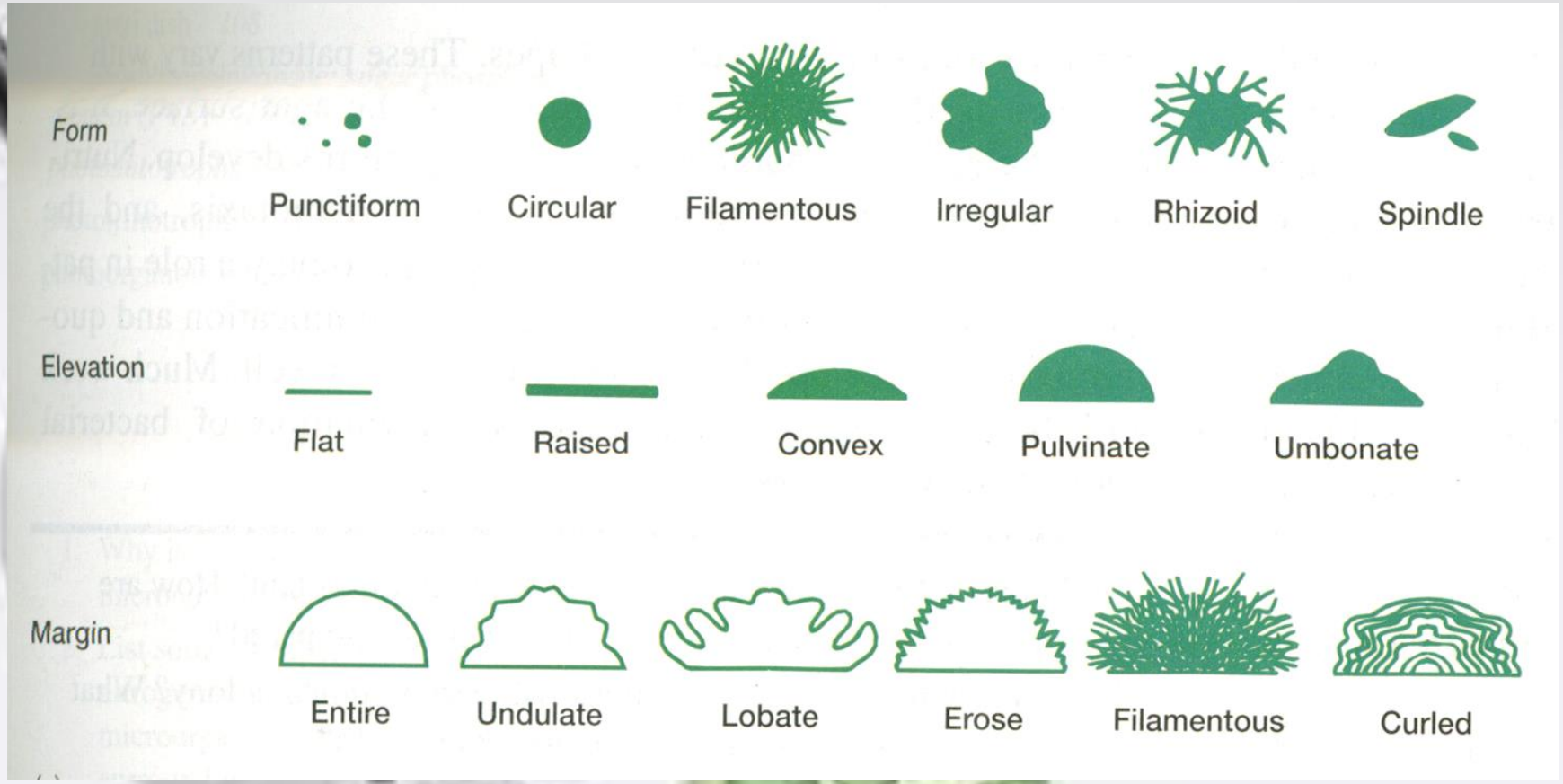
On solid media

1. Size of the colony
2. Shape of colony- circular, oval, irregular
3. Surface- smooth, shiny, rough, wavy
4. Margins- Irregular, regular
5. Edges- Entire, undulated, crennated, fimbriated
6. Consistency- Friable, butryous, viscid
7. Structure- Transparent, translucent, opaque
8. Colour
9. Emulsifiability
10. Satellitism
11. Medium changes in form of
    - Pigment production
    - Haemolytic reactions
    - Blackening

In liquid media

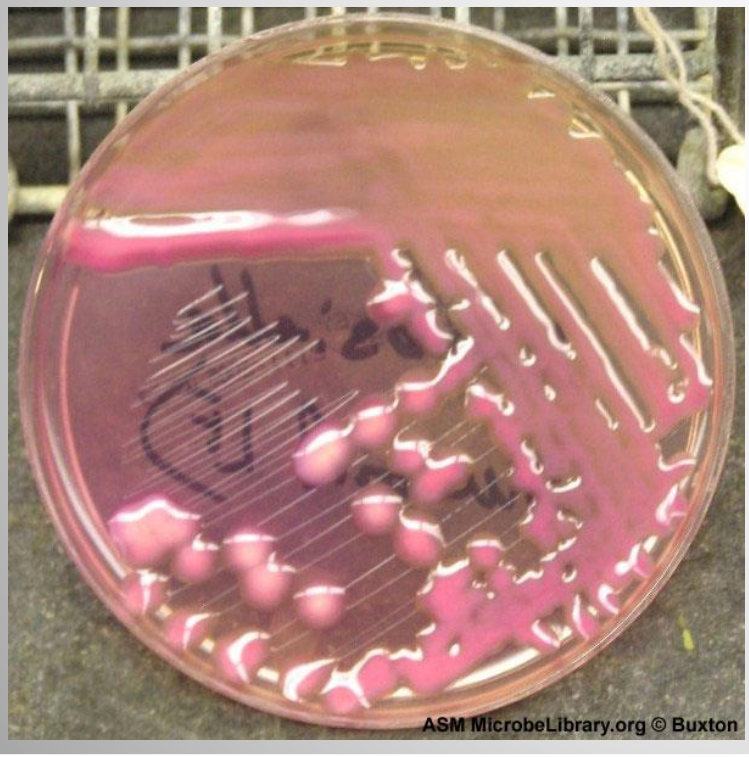
1. Presence of turbidity
2. Deposits- granular
3. surface growth as pellicle
4. Odour

Colony characteristics:



CONSISTENCY OF COLONIES

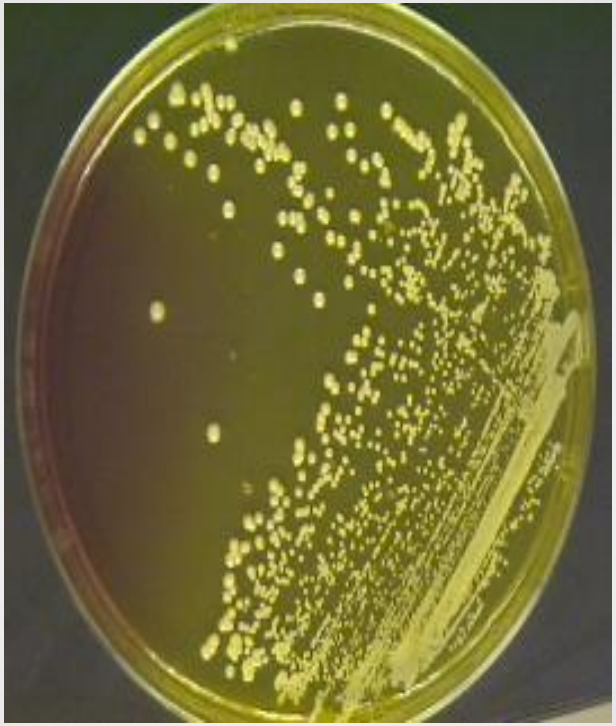
Mucoid



Rough

PIGMENTATION

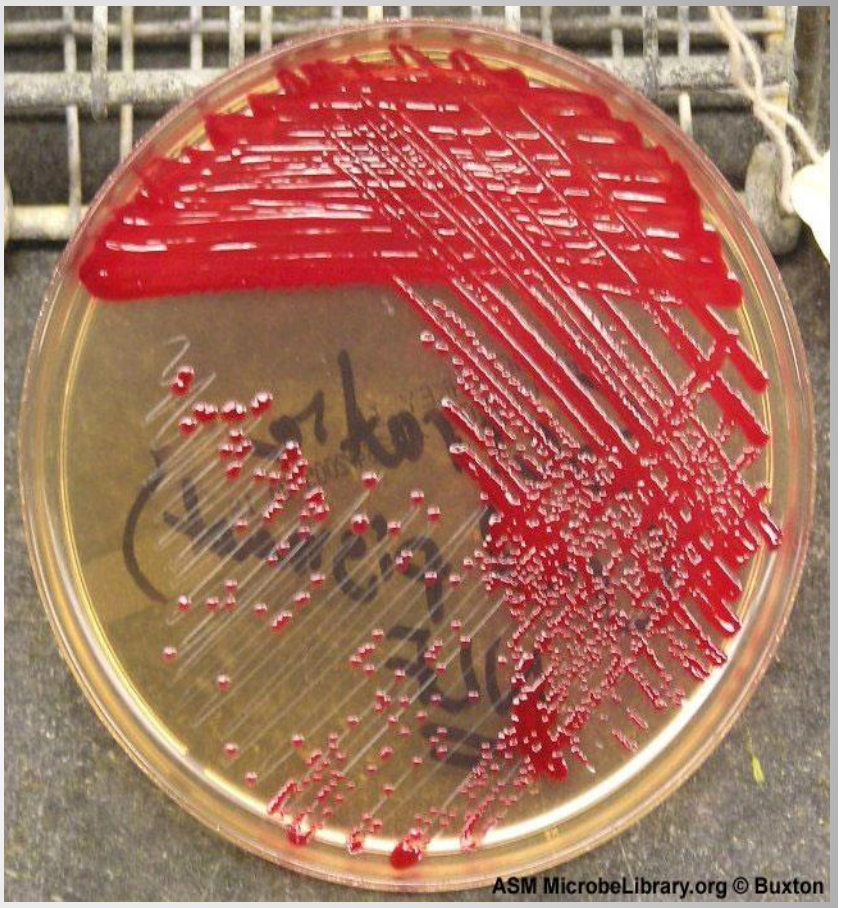
Staph. aures



Staph. citrus

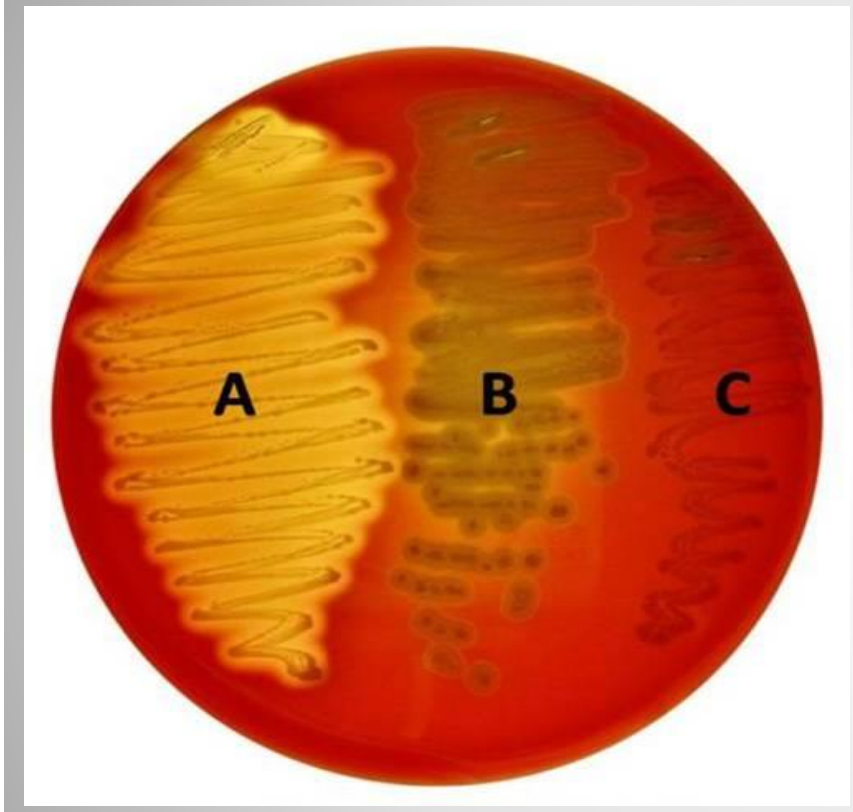
Staph. albus

Pseudomonas aeruginosa

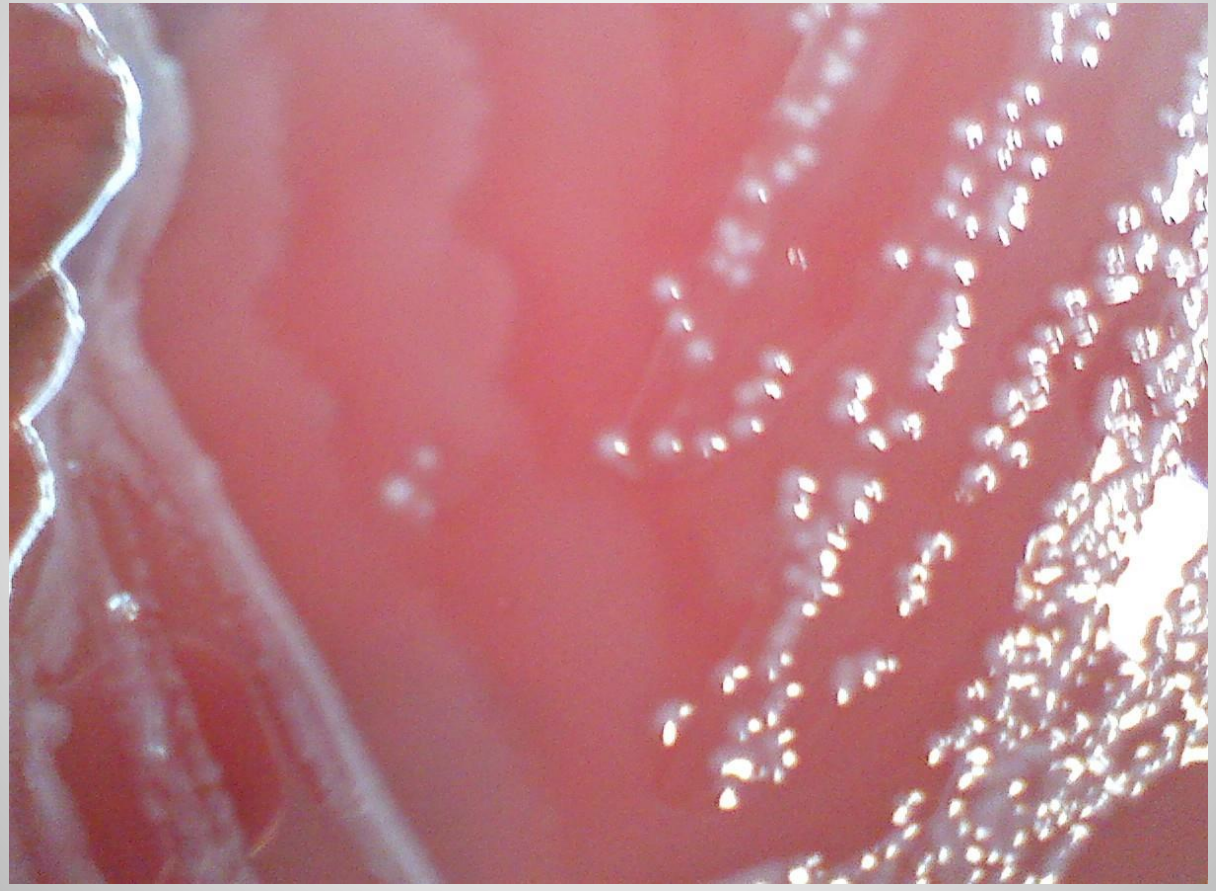


Serratia

HEMOLYSIS



SWARMING

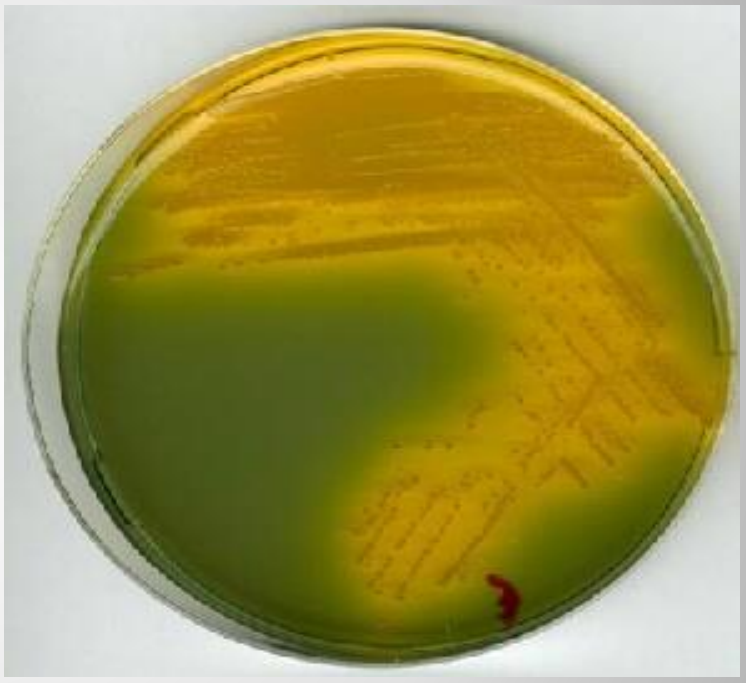


MacConkey agar

Tellurite agar

M023
S. aureus 25923

TCBS agar



Thank you