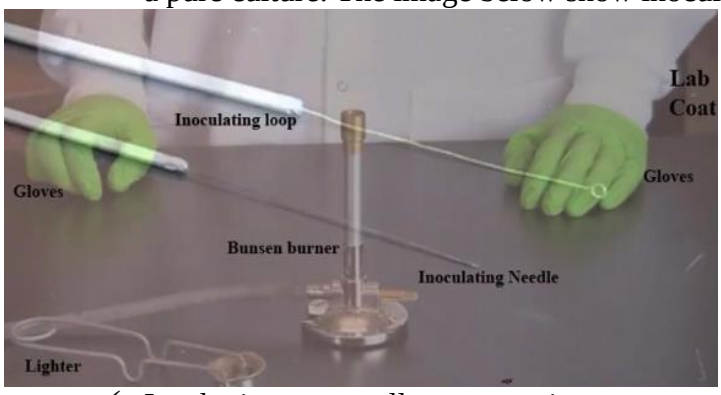
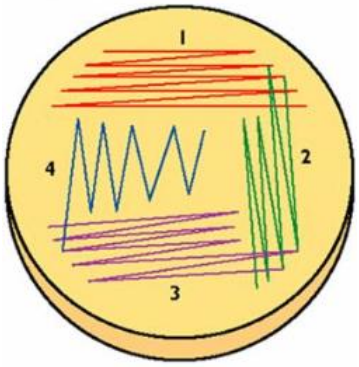
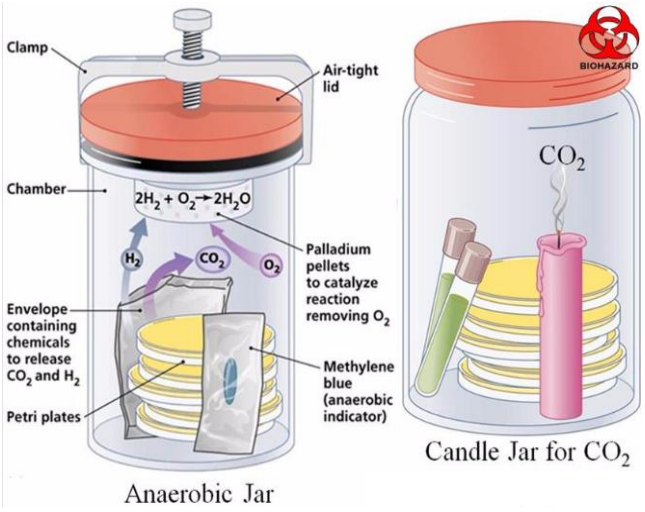
- **Culture:**
  - **It includes the following 5 stages in the process:**
    - ✓ Inoculation: this is represented by prevention of contamination when introducing a specimen (which is suspected to contain a microorganism) to a growth culture. Therefore, this aseptic technique will aid in the production of a pure culture. The image below show inoculation tools which are used:

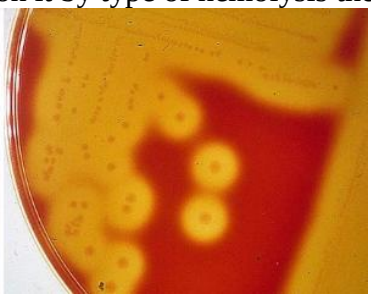




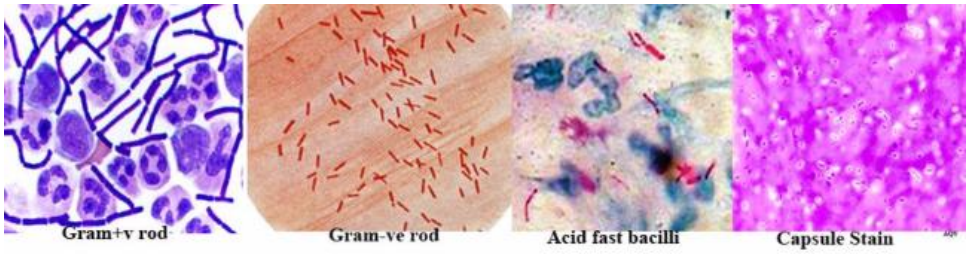
    - ✓ Incubation: to allow organisms to grow under optimal conditions (temperature: most organisms need a temperature between 35-37 C, O_2 and CO_2… etc).



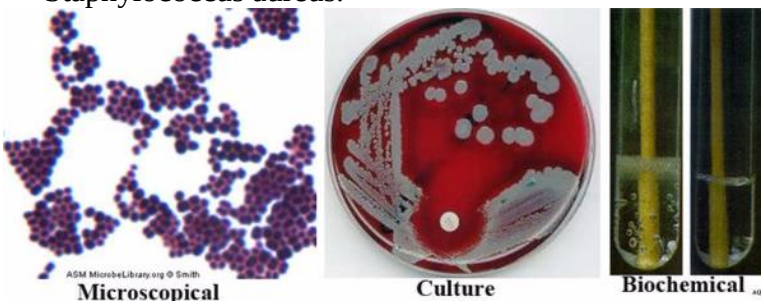
    - ✓ Isolation: notice that each colony on the surface of growth medium represents one organism. Isolation can done on:
      - ❖ *General growth media* (e.g. nutrient agar).
      - ❖ *Special growth media* (such as blood agar in which you differentiate between organisms growing on it by type of hemolysis they produce).



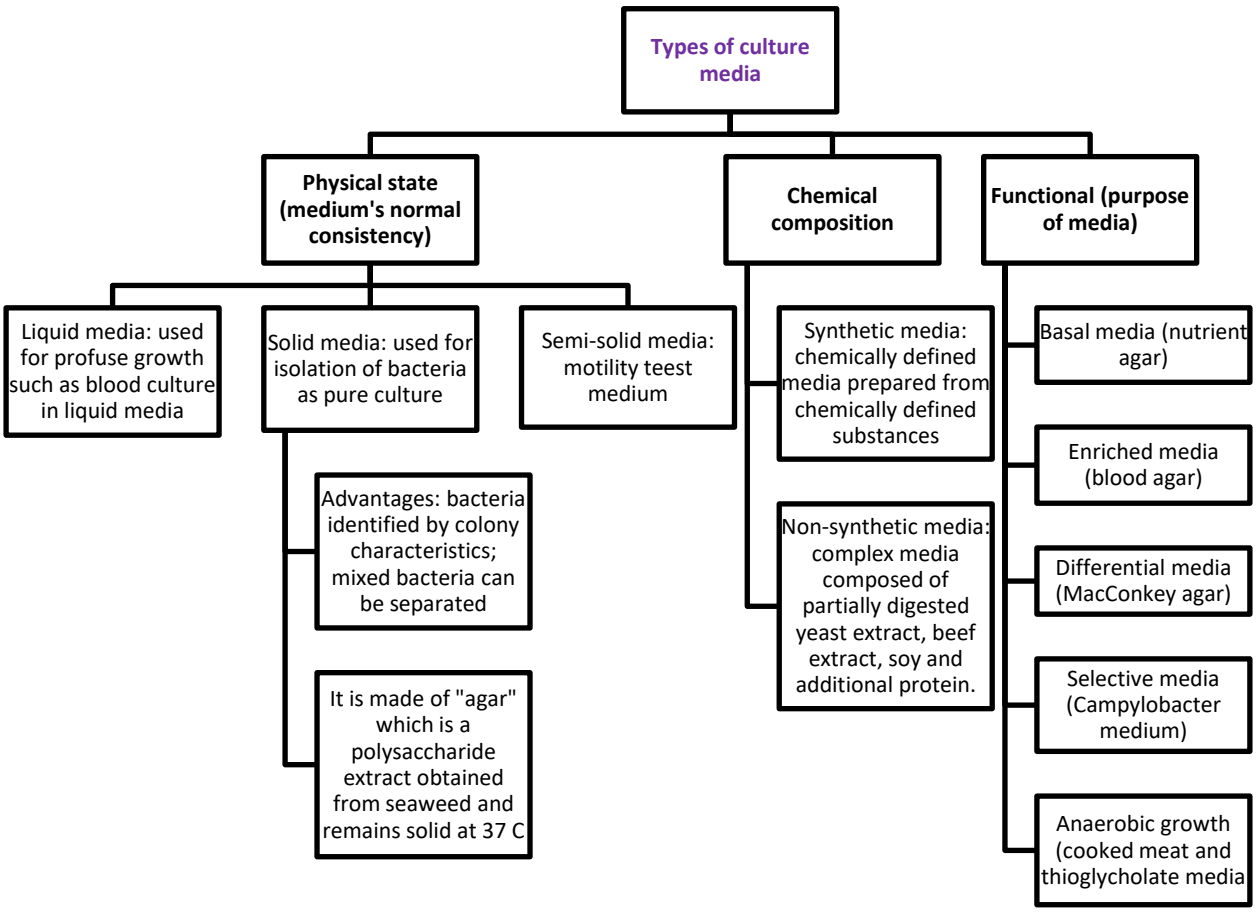
    - ✓ Inspection:
      - ❖ *Macroscopical*: observation and description of colony morphology.
      - ❖ *Microscopic*: wet mount preparation of stained smears. Smears can be stained with Gram's stain, Ziehl-Neelsen stain or capsule stain.



- ✓ Identification: correlating data from all observations and findings to identify organisms to species level.
  - ❖ *Example*: Gram (+) cocci, with grape-like clusters, golden-yellow colonies, Catalase (+) and coagulase (+) will identify → Staphylococcus aureus.



- **What are the types of culture media?**



- ✓ Basal media: used for growth of bacteria which don't need enrichment of media (e.g. Staphylococcus and Enterobacteriaceae). Example: nutrient broth, nutrient agar and peptone water.
- ✓ Enriched media: blood and other special nutrients are added to encourage growth of fastidious microbes. Examples: blood agar and chocolate agar.

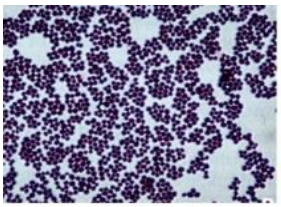
- ✓ Differential medium: allows you to distinguish between different microorganisms based on a difference in colony appearance (color, shape or growth pattern) on the medium.
- ✓ Selective medium: allows the growth of specific types of microorganisms while inhibiting others due to the presence of selective agents (such as dyes).

Yeasts: grow creamy-white colonies

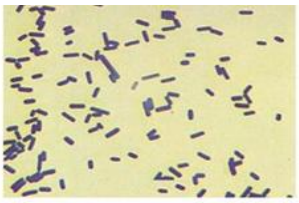
Molds: grow as filamentous colonies of various colors

- **Preparation of the smear:**
  - Small sample of bacterial culture is removed from the culture.
  - Bacterial suspension is smeared onto a clean glass slide.
  - Then, bacterial smear is dried slowly at first and then, when dry, heated for a few seconds to the point when the glass slide is too hot to handle.
- **Gran-staining technique:**
  - **Method:**
    - ✓ Fix the dried smear with methanol for 2 minutes.
    - ✓ Cover fixed smear with crystal violet stain for 30-60 seconds.
    - ✓ Rapidly wash-off the stain with clean water.
    - ✓ cover the smear with Lugol's iodine for 30-60 seconds.
    - ✓ Wash-off iodine with clean water.
    - ✓ Decolorize rapidly with acetone-alcohol. Wash immediately with clean water.
    - ✓ Cover smear with neutral red stain for 1 minute.
    - ✓ Wash-off the stain with clean water.
    - ✓ Wipe the back of the slide clean and place it in draining rack for the smear to air-dry.
    - ✓ Examine smear microscopically.
  - **Results:**

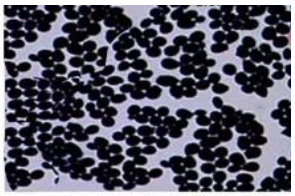
Gram (+) bacteria	Dark purple
Yeast cells	Dark purple
Gram (-) bacteria	Pale to dark red
Nuclei of pus cells	Red
Epithelial cells	Pale red



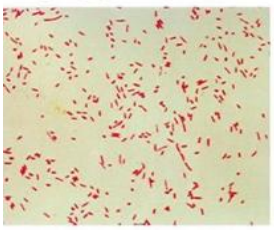
Gram positive cocci, e.g. *Staphylococcus aureus.*



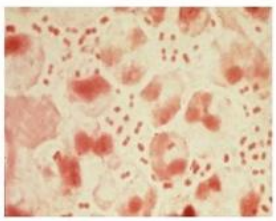
Gram positive bacilli (rods), e.g. *Clostridium species*



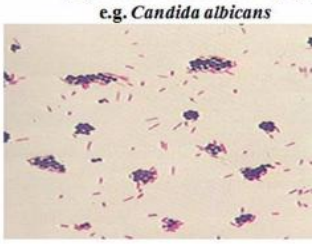
Gram positive oval yeast cells (big oval and rounded shapes), e.g. *Candida albicans*



Gram negative bacilli (rods), e.g. *Salmonella typhi*

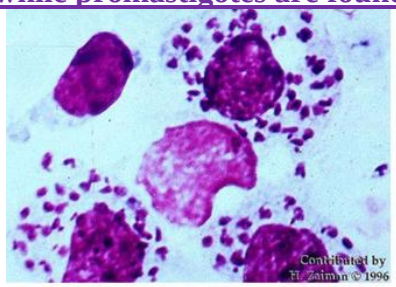


Gram negative diplococci cocci and polymorph white blood cells.



Mixed Gram positive cocci & Gram negative bacilli

- **The image below shows protozoa (Leishmania). Notice that amastigotes are found in macrophages while promastigotes are found in the vector (sandfly).**



Amastigote of *Leishmania* sp.

Promastigote of *Leishmania* sp.

- **Antibiotic sensitivity test:**
  - **Aim**: to determine which antibiotic will be most successful in treating a bacterial infection in vivo.
  - **Method used**: Kirby-Bauner.
  - **Process**: a small disk containing antibiotics are placed onto a plate upon which bacteria are growing → if bacteria are sensitive to the antibiotic → a clear zone of inhibition is seen around (indicating poor bacterial growth).