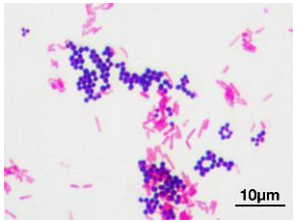
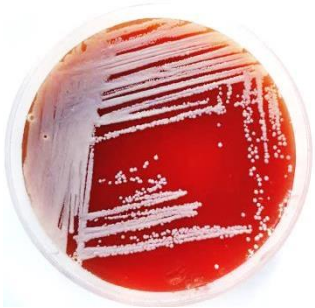


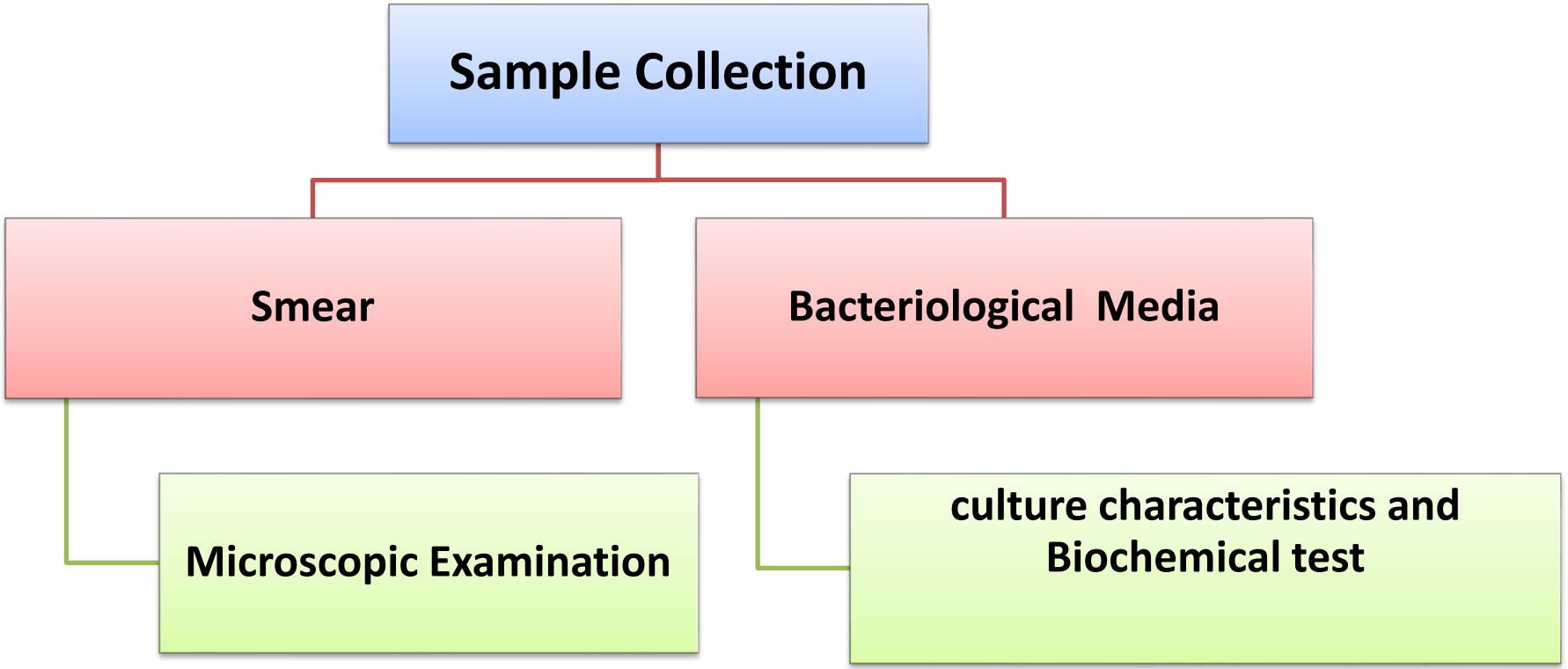
Clinical Microbiology

Hanan Yousef Jassim
Clinical Pathology
4th Class



- **Clinical Microbiology is to isolate and identify the pathogenic microorganism** since the veterinarian deals with different infectious organisms such as bacterial, viral or fungal organisms, so we must know how and when to take specimens, what examination can be done in the laboratory, and how to interpret the results. A veterinarian should be competent to perform simple techniques that will assist the diagnosis processes.

Bacteriological Examinations and Diagnosis



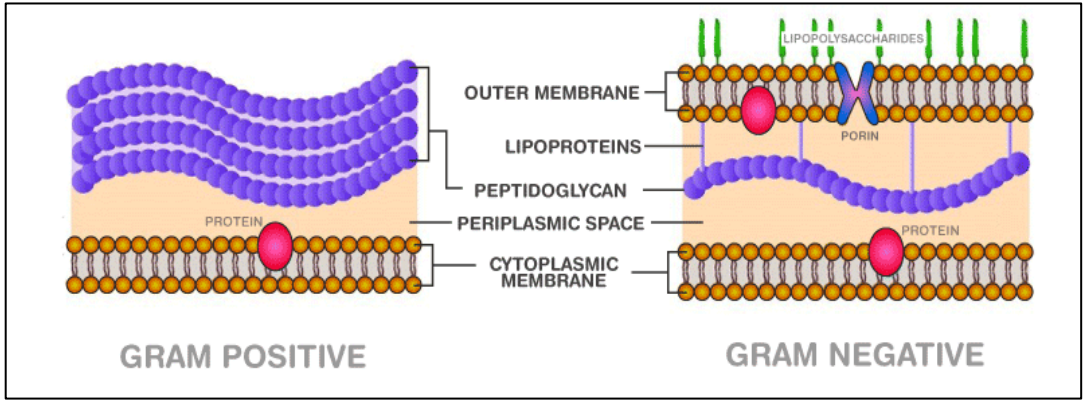
Smear

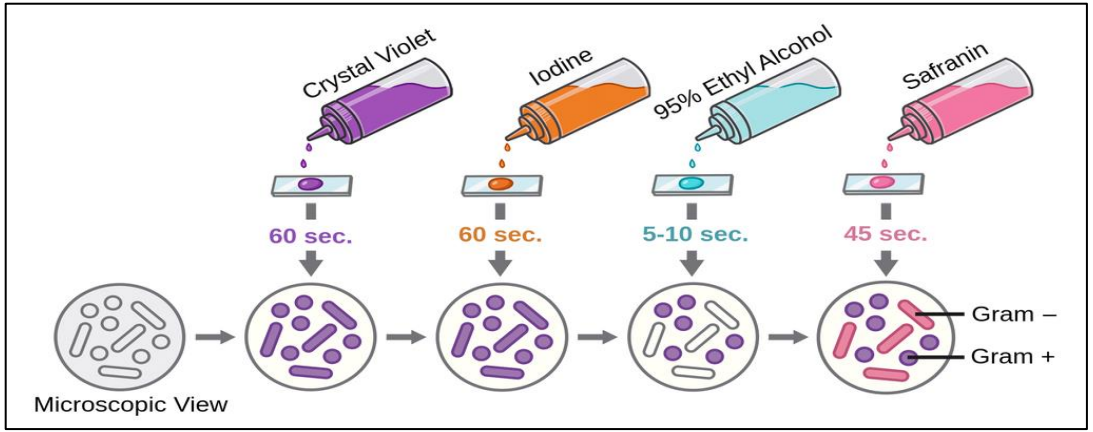
- The fixed smears are placed on a staining rack over a sink.
- The staining solutions are flooded over the entire smear and left on the slide for the appropriate time.
- Between each staining reagent the smear is washed under a gently running tap, excess water tipped off and the next reagent added.
- Finally the stained smear is washed and air-dried.

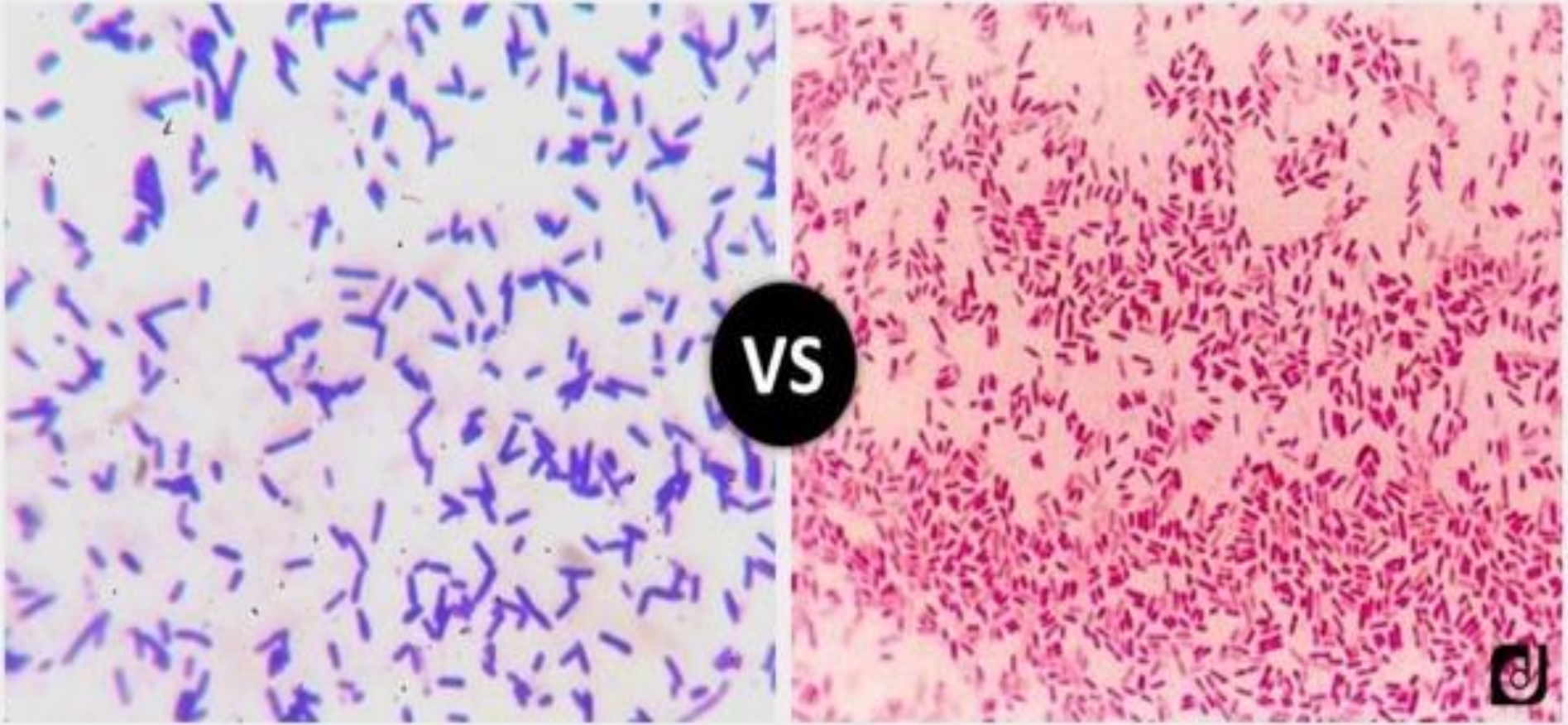
Common stains used for microbiological diagnosis

1. Gram stain

Gram stain used to differentiate between gram positive and gram negative bacteria since gram positive bacteria will retain the crystal violet iodine complex and stain purple-blue, whereas gram negative will be decolorized and stained red color by the counter stain.



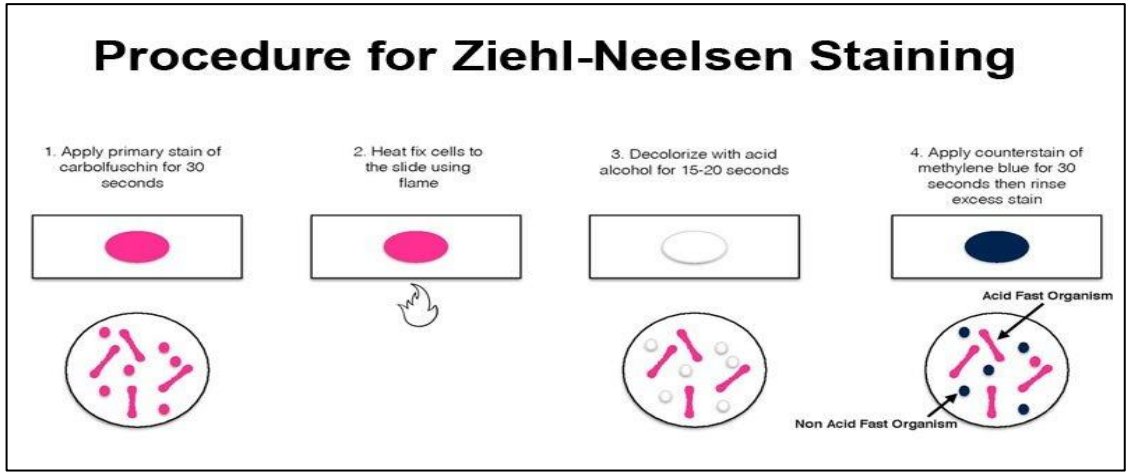


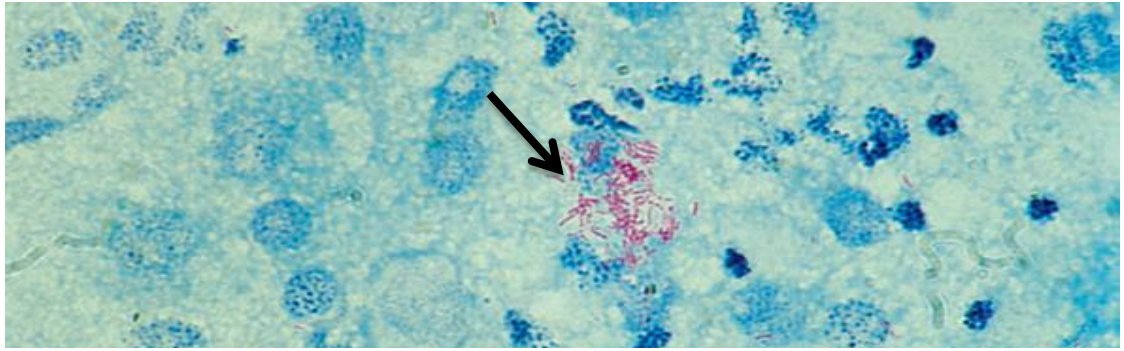


Gram Positive Bacteria vs. Gram Negative Bacteria

2. **Ziehl-Neelsen stain (acid-fast stain)**Its commonly used to Bacteria whose positive to Ziehl-Neelsen stain, such as *Mycobacterium* spp., which stains bright red. The background and other bacteria stain with blue color.

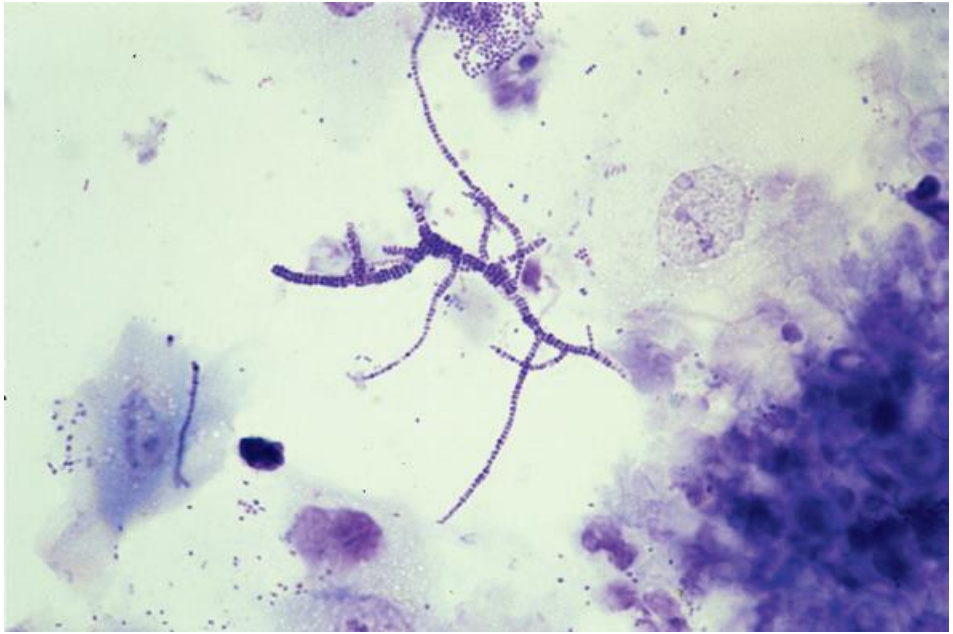
3. **Methylene blue stain:**
This stain is used specially for *Pasteurella* spp. which characterized by bipolarity shape .





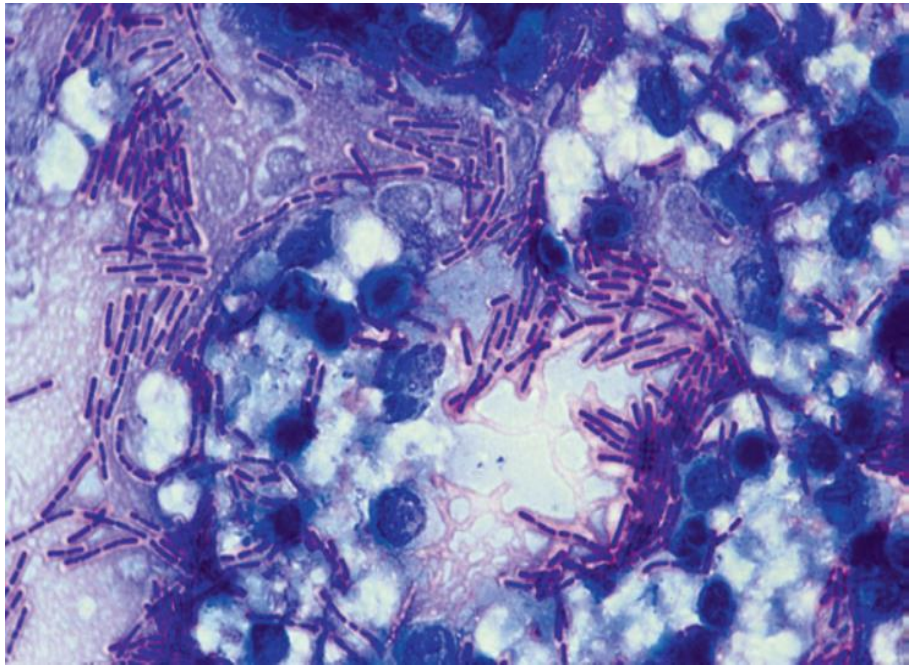
Ziehl-Neelsen stained smear from a tuberculous lesion. red Ziehl-Neelsen positive thin rods of *Mycobacterium*

4. **Giemsa stain:** This stain used to stain *Bacillus anthracis* capsule, *Mycoplasma haemofelis*, *Borrelia anserina* and *Dermatophilus congolensis*



*Giemsa-stained smear from ovine scab material Shows *Dermatophilus congolensis*

5. **Polychrome Methylene Blue Stain:** This stain is used specially for Bacillus anthracis which is stained blue, and the capsular material stained with pale pink color.



*Polychrome methylene-blue-stained bovine blood smear showing capsulated *Bacillus anthracis*. Note the square-ended rods in short chains with pink capsule surrounding the blue cytoplasm

Bacteriological Media

Diagnostic bacteriological media can be divided into the following :

1. **Basic nutritive media:** These are capable of sustaining growth of the less fastidious bacteria. Example: **Nutrient agar**.

2. **Enriched media "agar media":** These are used for fastidious bacterial growth, and usually enriched with blood, serum, or egg yolk. Example: **Blood agar** .

3. **Enrichment broth:** Liquid media that are selective for a particular bacteria, such as for the selection of the salmonella.

4. **Selective agar media:** This media used for the growth of a particular bacterium or group of bacterium, and are used extensively in diagnostic bacteriology. They contain inhibitory substances that prevent the growth of unwanted bacterial species. Example: **MacConkey agar**.

5. **Indicator (differential) media:** These are particularly useful for diagnostic bacteriology. Example: **Edwards medium.**

Examples of Culture Media

MacConkey agar/ Selective and Deferential

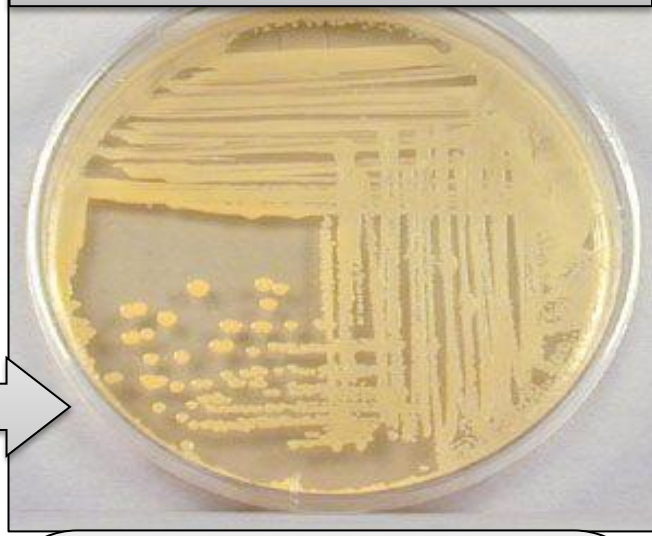
- Isolate Gram-negative and differentiate them based on lactose fermentation. Lactose fermenters turn red or pink, non fermenters do not change color.
- The media inhibits growth of Gram-positive organisms with crystal violet and bile salts
- pH indicator phenol red

Blood Agar/ Enriched media

- Isolate fastidious organisms and detect hemolytic activity
- Contain 5-10% Sheep Blood

Mannitol salt agar/ Selective and Deferential

Nutrient agar/nutritive media



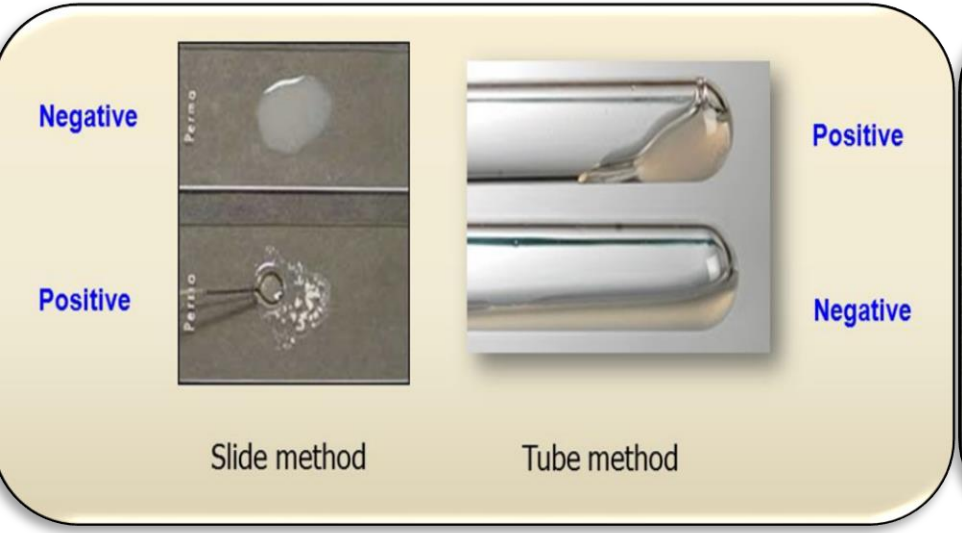
Examples of Culture Media

- Contains a high concentration (7.5–10%) of salt (NaCl) there for considered selective for some Gram-positive bacteria that tolerate high salt concentrations.
- It is also a differential medium for mannitol-fermenting staphylococci, containing carbohydrate mannitol
- pH indicator phenol red.

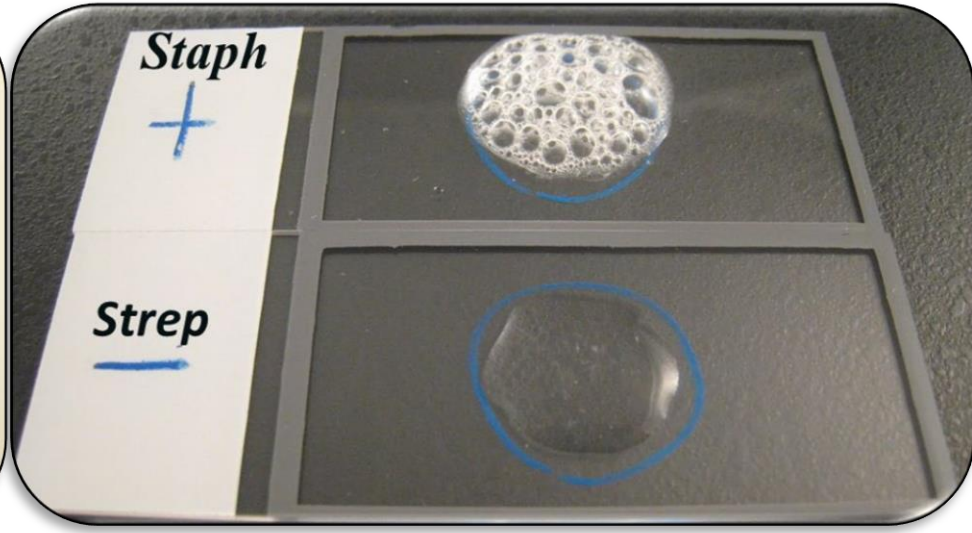
- General purpose, nutrient medium support the growth of a wide range of non-fastidious organisms.

Biochemical Test

1) Coagulase Test

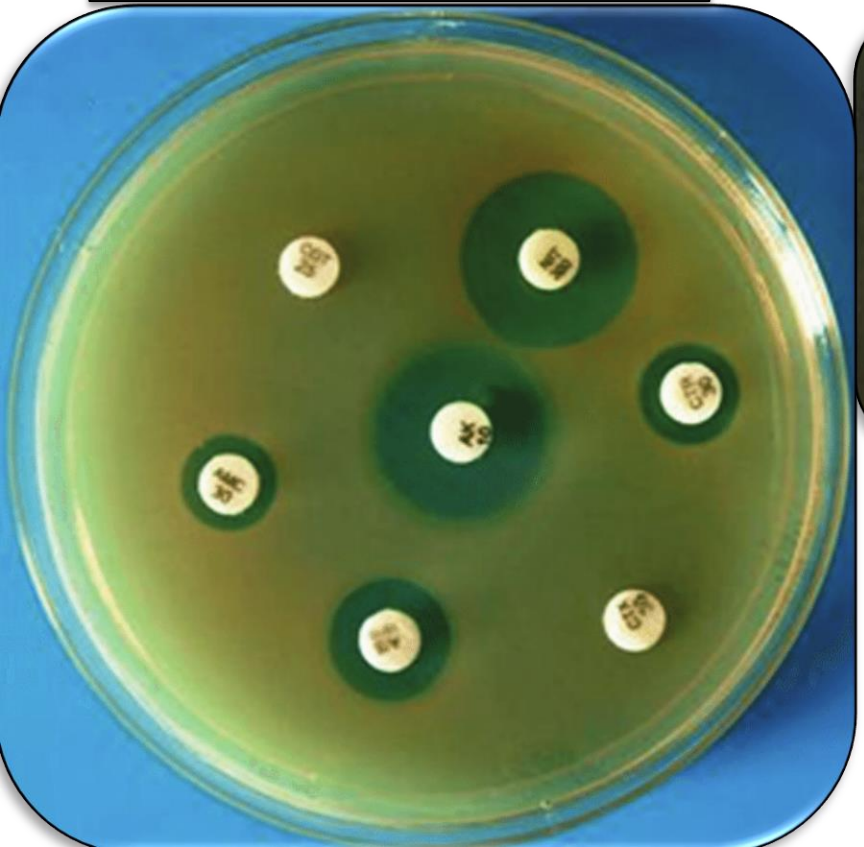


2) Catalase Test

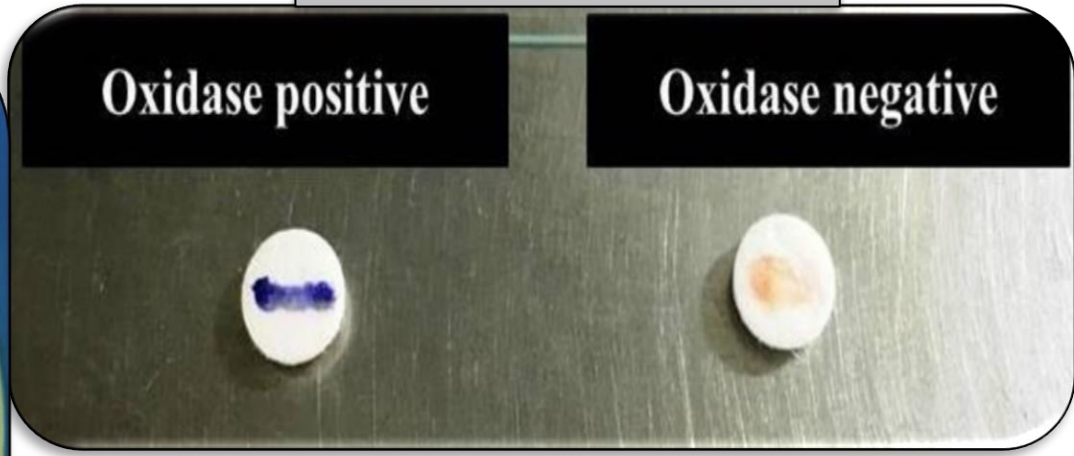


Biochemical Test

3) Antibiotic Sensitivity Test

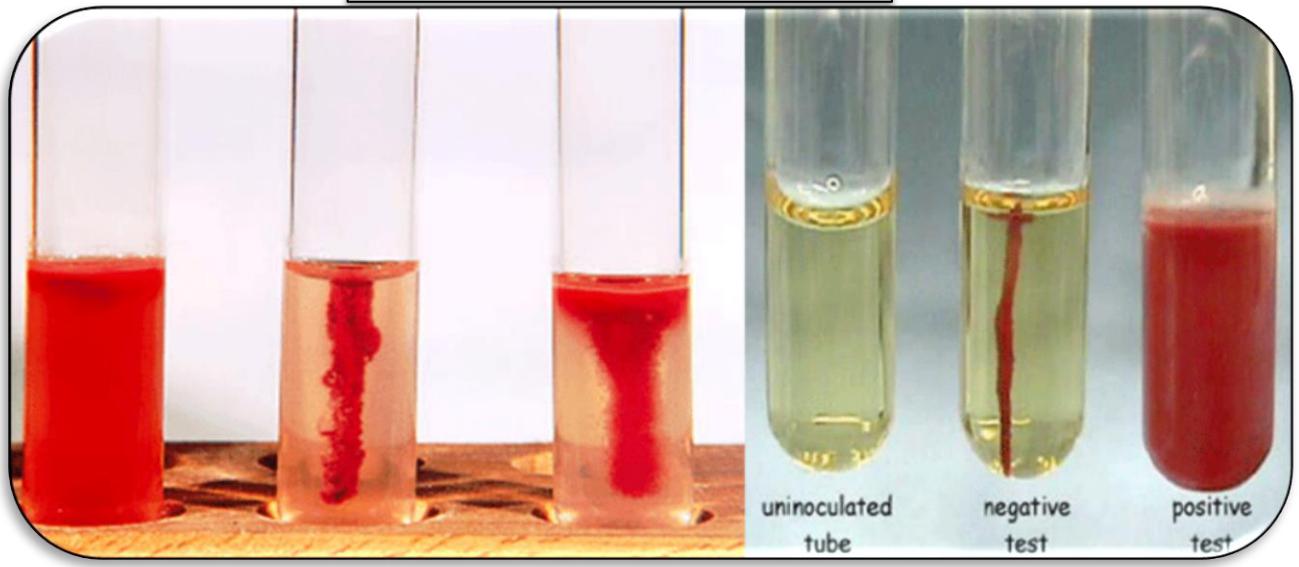


4) Oxidase Test



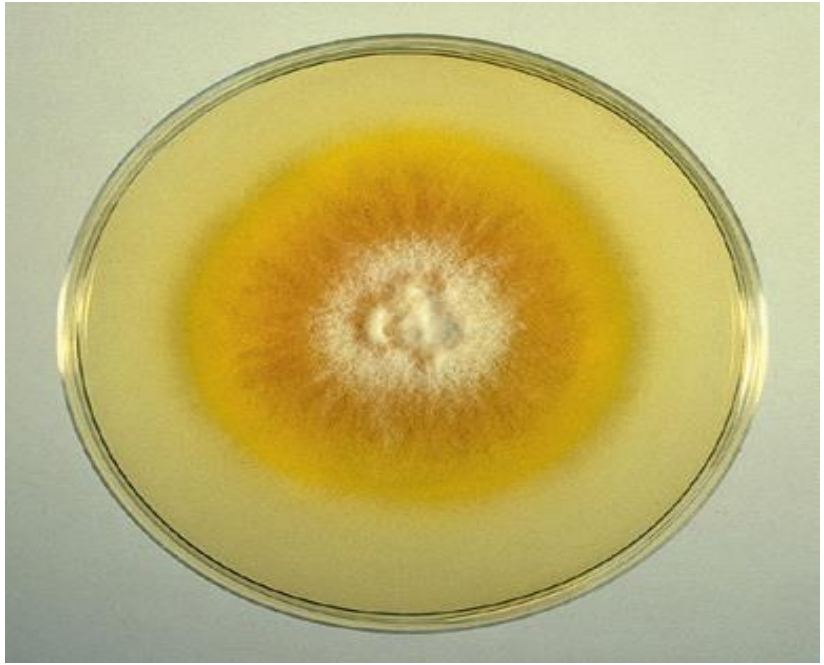
Biochemical Test

5) Motility Test



Fungal Examinations and Diagnosis

- **10–20% KOH** wet preparations Clears specimens to make fungi more visible.
- Examine under low and high-dry objectives or phase contrast Fungal elements of most moulds and yeasts. Dimorphic fungi as yeast-like forms in tissue. Arthrospores on affected hairs for dermatophytes

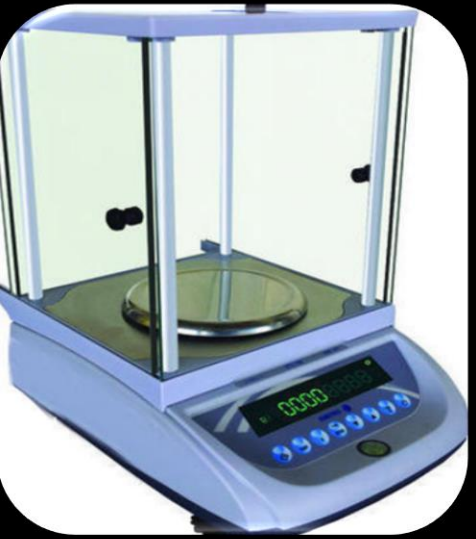


Microsporum canis **on Sabouraud agar, 10 days**

preparation of culture media

Equipment and Instruments

Electronic balance



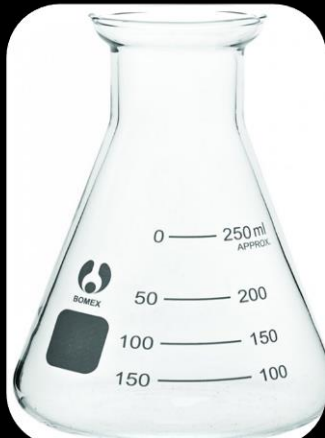
Autoclave

Culture media base

Distal water

Petri dishes

Conical Flask



Microbiological Hood

Incubator

Inoculation loop