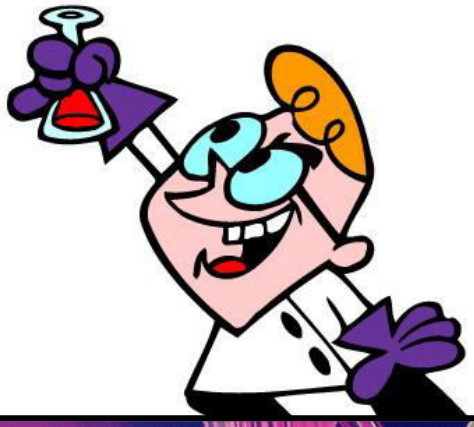


Clinical Microbiology

1- SAFETY PRECAUTIONS IN THE MICROBIOLOGY LABORATORY

I – PPEs (personal protective equipment):

Wear a lab coat, rubber gloves, goggles, mask and foot wear while at work.

II-Personal Hygiene

- Do not eat, drink or smoke in the lab.
- Do not lick labels.
- Do not put fingers, pencils or other objects into mouth.
- Do not finger the eyes.
- Wash hands with soap and water before leaving the lab.
- Laboratory personnel should be vaccinated against infectious agents.

❖ **Washing hands with water and soap or other detergents containing antiseptic agent (more effective) But If visible dirt on the hands, you must wash with water and soap.**

III- Safe work practice

- Wipe benches with a disinfectant before and after work.
- Never pipette by mouth.
- Label all tubes, plates and any lab container.
- Be aware of chemical hazards
- Be careful near Bunsen flame
- Treat all organisms as possibly **infectious**.
- If dealing with a highly contagious organism work should be performed under **a laminar flow hood** or **glove box.**

Biological safety cabinet

IV- Administrative control

- Restrict access to authorized persons and lab personnel.
- Do not take cultures out of the lab.
- Keep doors always closed.
- Report any accidents, spills, broken glass, cuts and injuries (needle sticks).

V-Adequate waste disposal

- All biohazardous materials should be packed in such labeled containers.
- Sharp contaminated objects (syringe, needles, broken glass) placed in a puncture proof container (safety box) until disposal.

Waste disposal Bags

Use : Disposal of biological waste

Biohazard sign

Use : labeling of infectious materials

Safety Box

Use : Disposal of needles and sharp objects

2- MICROSCOPE

Bacteria are very small in size, measured in term of microns (μ). they cannot be seen by naked eye.

Microscope is an instrument for producing magnified and resolved image of objects that are too small to be seen by the naked eye.

Factors determine the quality of an optical image:

1-Magnification:

- The ability to increase the size of an image by using a convex lens.

2-Resolution:

- The ability to distinguish two neighboring points as separate entities allowing the study of the structural details.
- Depends on **the wavelength** of the used source of illumination (shorter wavelength → the greater the resolution).

TYPES OF MICROSCOPES

A-Light microscopes

I- Using visible light

- Bright-Field Microscope.
- Dark-field microscope.

II- Using ultraviolet rays

- Fluorescent Microscope.

B-Electron Microscopes

-Scanning electron microscope
-Transmission electron microscope

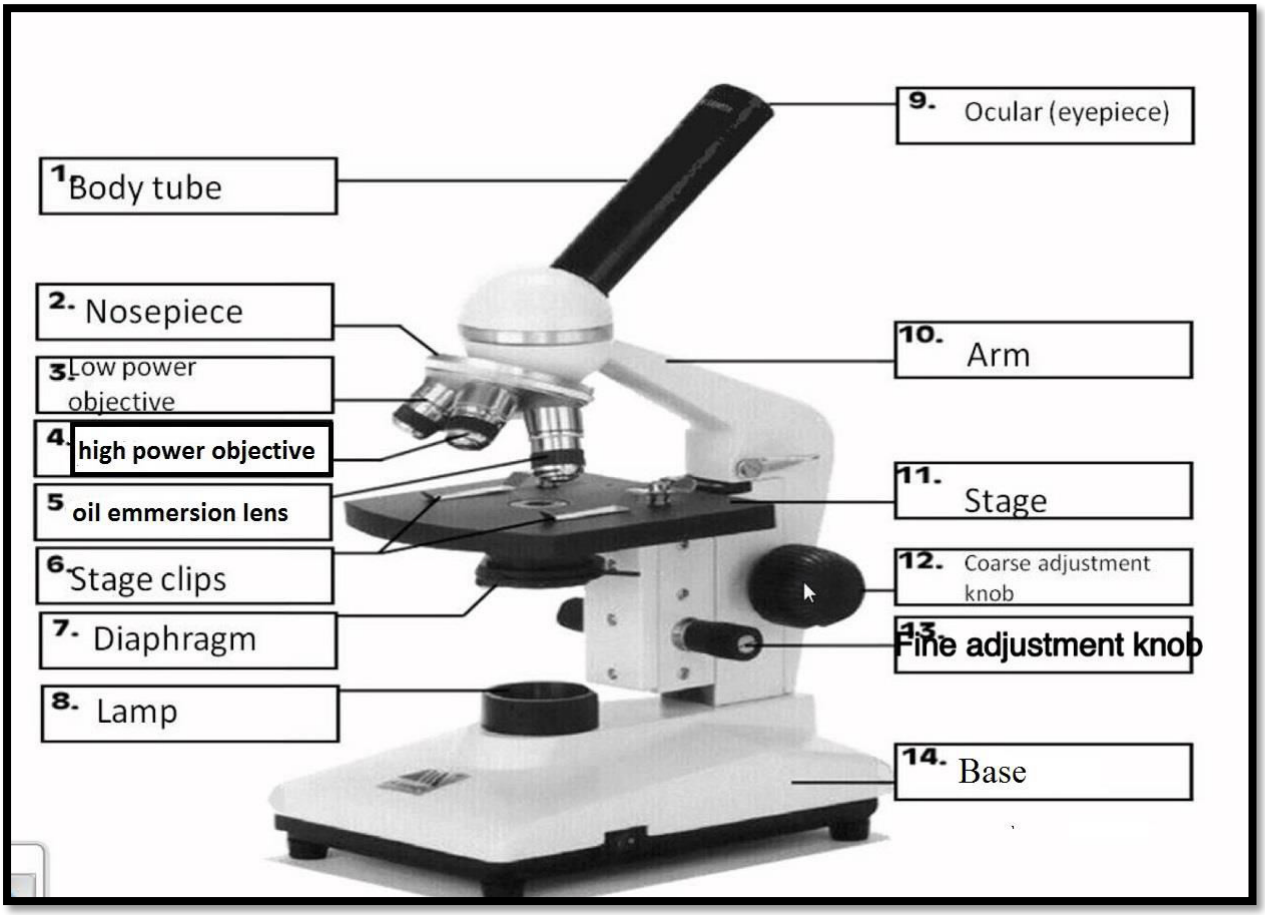
Bright-Field Microscope

(Ordinary light microscope)

Most widely used.
It produces a dark image against brighter background.

Components of light microscope:

- Support system
- (Magnification) system
- Illumination system
- Adjustment system



❖ **Objective lens:**

- **Low Power Objective (10 x):** Find the object.
- **High Power Objective (40 x):** Focus the object.
- **Oil Immersion Objective Lens (100 x):** Fine focus.

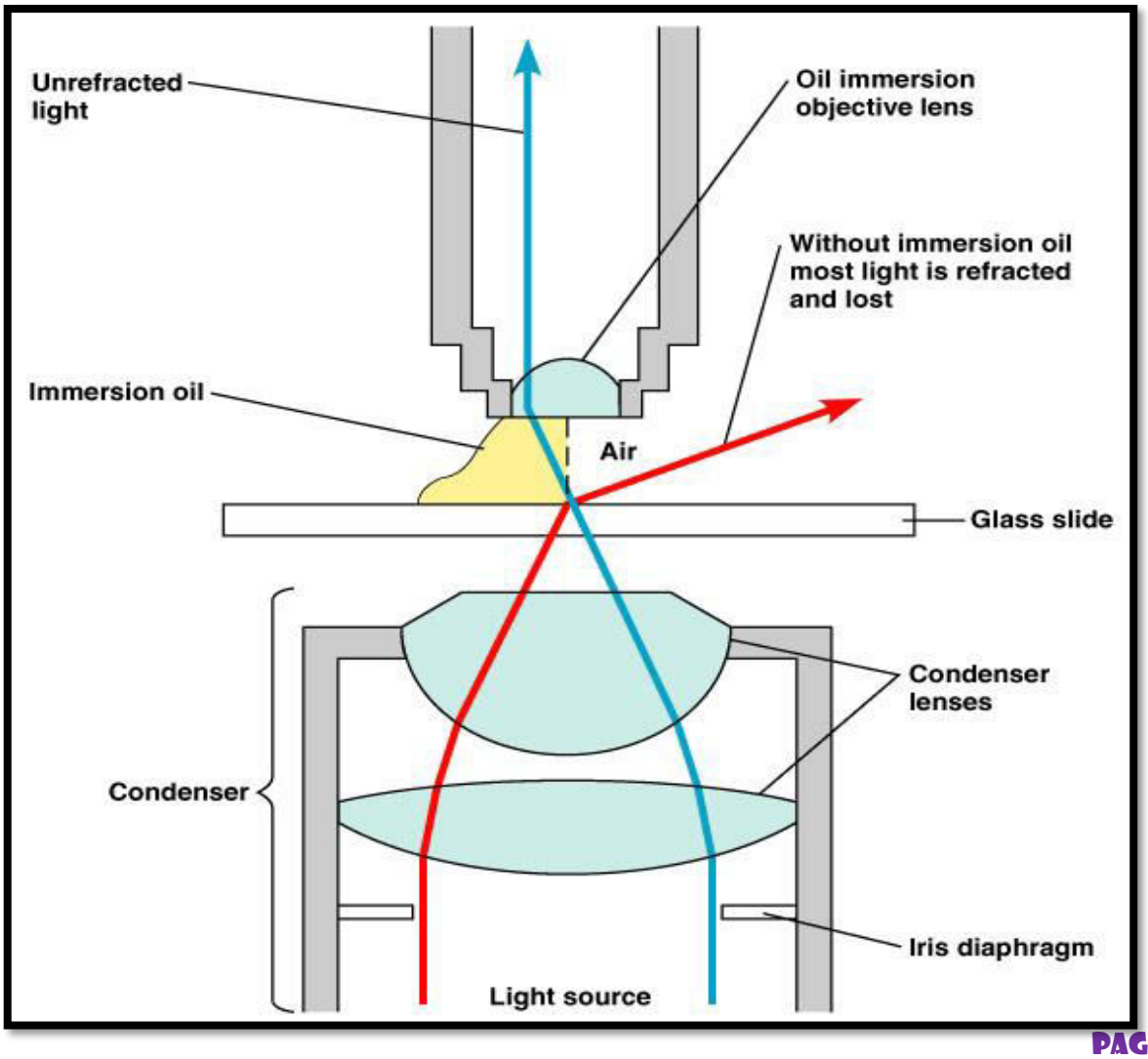
❖ **Eye piece:** The lens that you look through (**magnification power** = 10)

Total Magnification = Ocular power X Objective lens power.

Ex. Magnification of microscope when using low power lens:
10x10 = 100 times

Value of using oil emersion lens

Space between object and oil emersion lens filled with cedar – wood oil having the same RI of the glass slide and lens thus the rays will not be refracted and will pass into the objective and a bright image is seen.

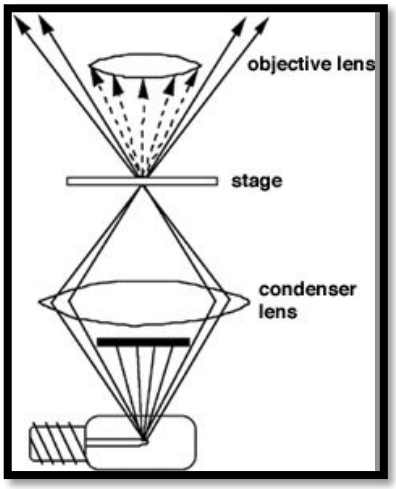


Dark-Field Microscope

Principle

- Object appears as bright against **dark background**.
- Special condenser is used that allows oblique peripheral rays only to pass to the object.

These oblique rays do not enter the objective lens and do not reach the eye unless they are scattered by objects.



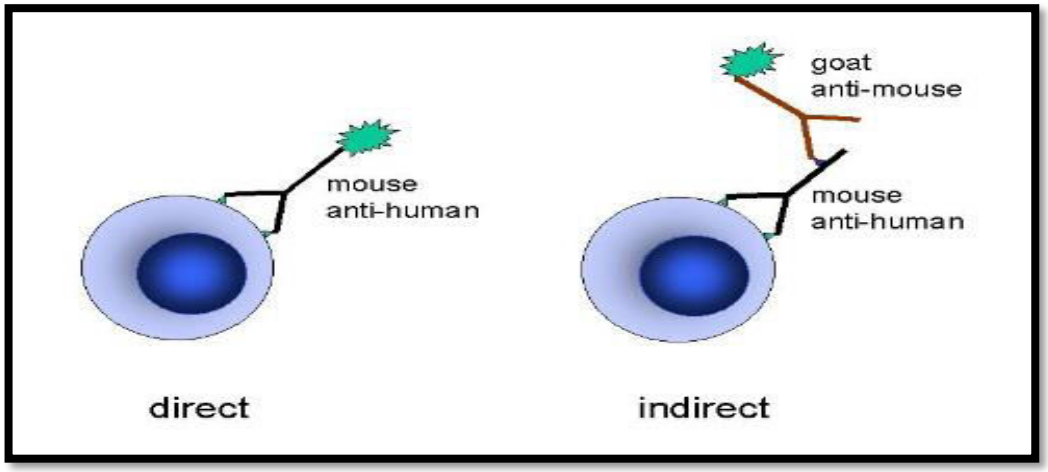
Fluorescent Microscope

Principle

- **Source of illumination**: **UV light.**
- **Specimens** stained with **a fluorescent dye**.
- When dyes exposed to **UV** they convert this invisible short wavelength radiation into visible longer wavelength radiation.
- dye becomes luminous and are said to be **fluoresce**.

Techniques

- **Fluorochroming**: direct interaction between fluorescent dye and target
- **Immunofluorescence**: fluorescent dye is linked to specific antibody to target



3- LABORATORY DIAGNOSIS OF BACTERIOLOGICAL INFECTIONS

The laboratory investigations for diagnosis of microbial diseases involve:

1. Specimen collection.
2. Microscopic examination.
3. Cultivation.
4. **Identification by:**
   - Culture morphology.
   - Gram-stained film.
   - Biochemical reactions.
5. Typing.
6. Serological identification.
7. Molecular methods.
8. Animal pathogenicity.

I- Specimen collection

General rules for collection and transportation of specimen

- specimens must be handled with care.
- Specimens should be collected before starting treatment with antibiotics.
- Sufficient material should be collected.
- Avoid contamination of specimens.
- All specimens should be properly labeled and should be associated with a ***request form*** **that includes:**

1. Patient's name.
2. Age.
3. Sex.
4. Source and time of collection.
5. Suspected diagnosis.
6. Antimicrobial treatment taken.
7. The test requested.
8. The referring physician.

Transport of microbiological specimens

- All specimens must be transported **immediately** to lab without any delay.
- if there is a delay use **a transport media** for transport (**Cary Blair** transport media for **stool** specimens and **Boric acid** for **urine** specimens).

II-Microscopic examination

Two methods are used for microscopic examination of bacteria:

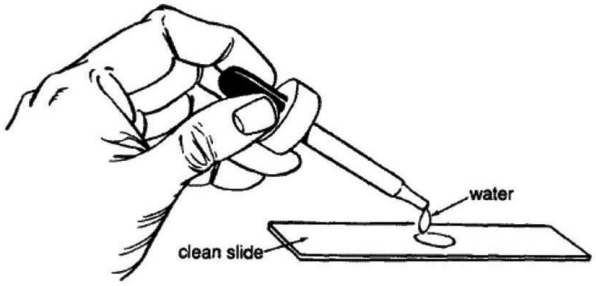
1- **Unstained preparation**
2- **Stained preparation**

1-Unstained Preparations

- ✓ It studies morphology and motility of bacteria in living free form.

Wet mount:

- ➢ Place a small drop of the bacterial suspension onto a glass slide.
- ➢ Apply a cover glass to the drop.
- ➢ Examine under the microscope.



2-Stained Preparations

- ✓ **Staining** is coloring of microorganisms with **a dye**.
- ✓ Before staining **a Smear** of bacteria is done which is a thin film of specimen or culture is spread over a slide
- ✓ Staining is important to accurately study Morphology and Staining reaction of bacteria.

Staining techniques

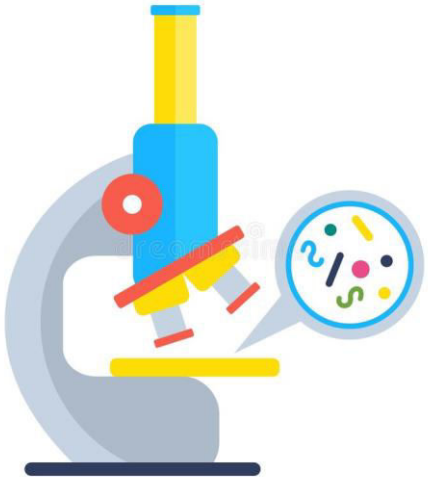
1. **Simple staining**
2. **Differential staining**
3. **Special staining**

Simple staining

- ✍ Only **a single stain** is used.
- ✍ This stain show morphology of organism and cellular elements in exudates.

Differential staining

- **More** than one stain is used.
- React differently with different types and parts of bacteria.
- **It usually involves:**
  1. Primary stain
  2. Mordant (**Fixative**)
  3. Decolorizing agent
  4. Secondary (**Counter**) stain



Commonly used stains are:

* **Gram Stain**
* **Ziehl-Neelsen Acid Fast Stain.**

Gram staining

- It is the most useful staining procedure in medical microbiology.
- It differentiates *bacteria into **Gram positive** and **Gram negative** based on differences* in **cell wall structure**.

- **Primary stain**: **Crystal violet**
- **Mordant**: **Iodine**
- **Decolorizing agent**: 95% alcohol
- **Counter stain**: **carbol fuchsin** or **Safranin**

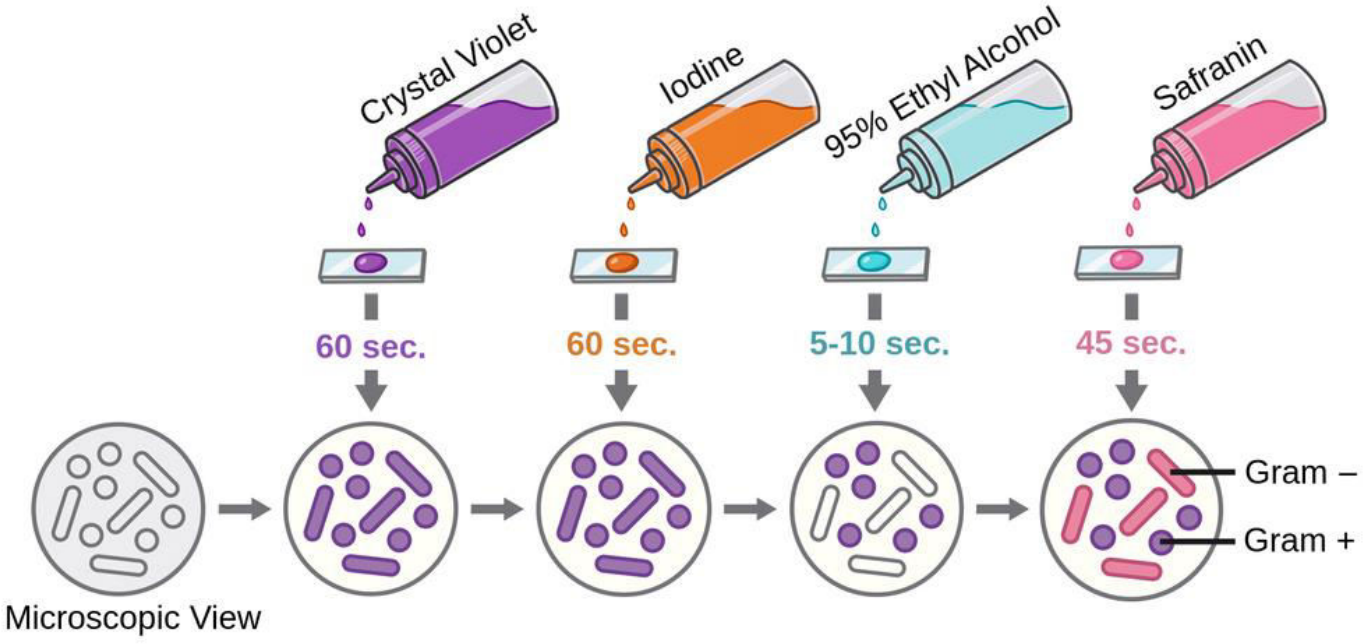
Procedures:

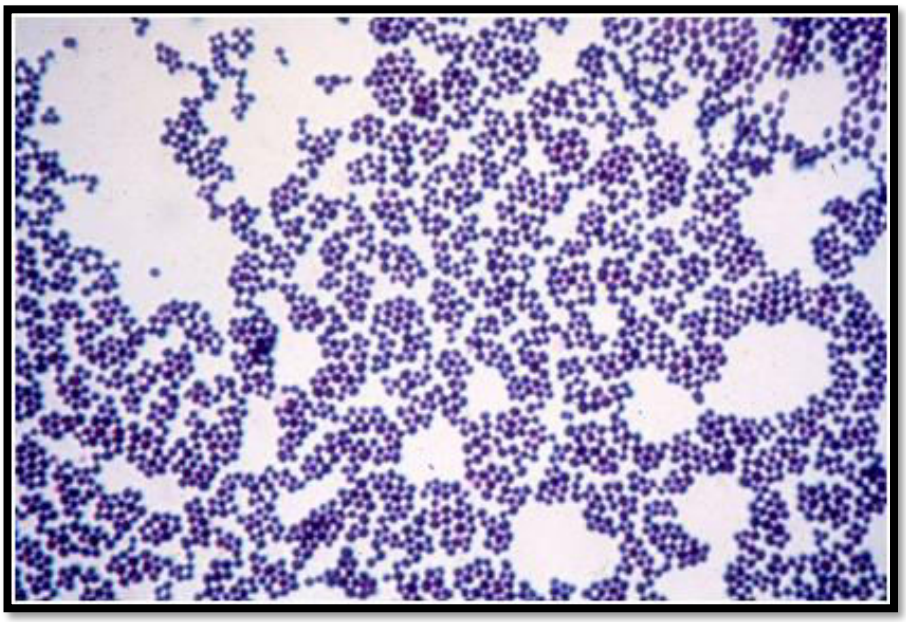
1. Flood the smear with crystal violet for about 30 seconds.
2. Pour off the crystal violet.
3. Add fresh iodine and leave it for 1 minute.
4. Decolorize by adding **95% alcohol**.
5. Wash with water
6. **Counterstain**: with dilute carbol fuchsin for 30 seconds.
7. Wash with water.

Results:

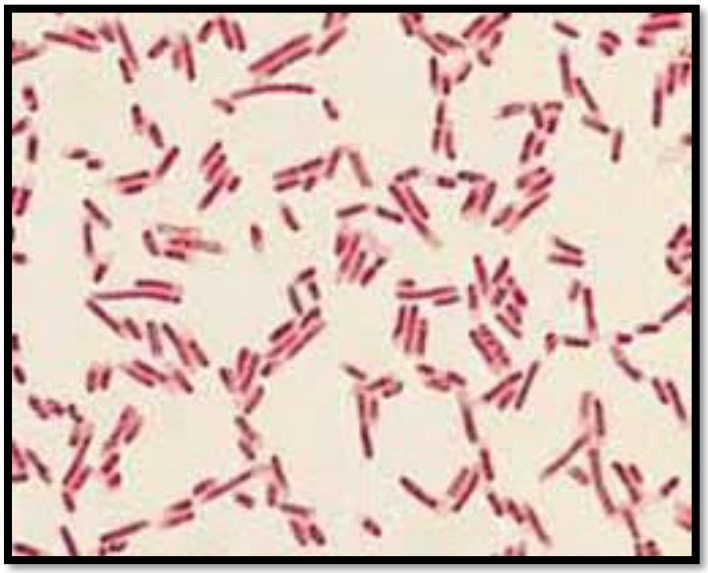
Gram positive bacteria: appear violet color cocci or bacilli.

Gram negative bacteria: appear red color cocci or bacilli.





Gram positive cocci



Gram negative bacilli

Ziehl-Neelsen Acid Fast Stain

- Used to identify **Mycobacteria**.
- The cell wall of the Mycobacterium contains high lipid (**mycolic acids**) content making them **unable** to be stained by Gram stain.

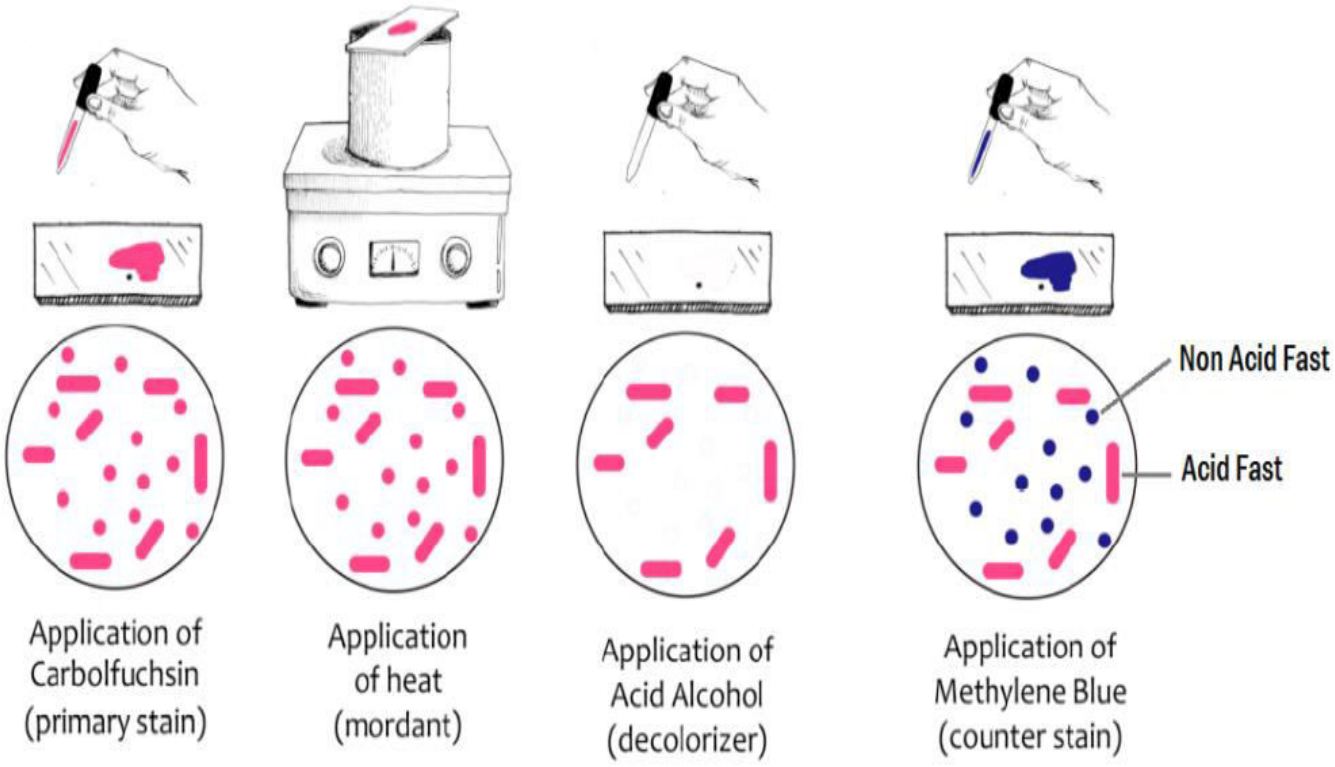
- **Primary stain with mordant: Conc. Carbol fuchsin** in phenol.
- **Decolorizing agent: 2**5 % H2SO4 and 95%alcohol **or** 3% HCL in 95%alcohol
- **Counter stain**: **Methylene Blue.**

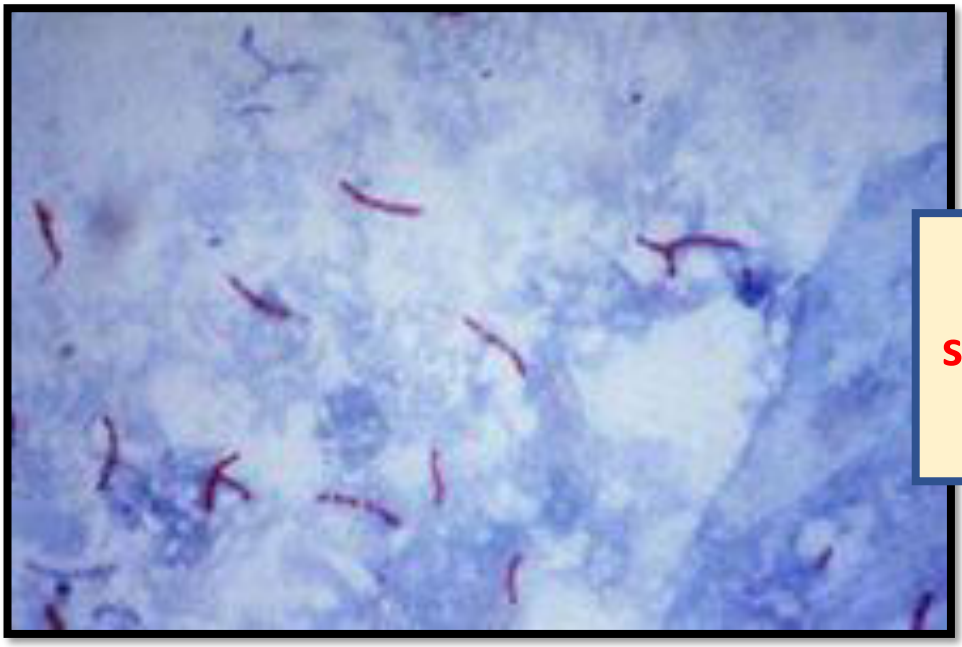
Procedures:

1- Flood the smear with strong carbol fuchsin for about 5-10 min with heating.
2- Wash with water.
3- Flood the slide with 20% H2SO4 for 1 min.
4- Wash with water.
5- Flood the slide with 95% alcohol for 2 min.
6- Wash with water.
7- **Counterstain**: with methylene blue for 2 min.

Results:

Acid fast bacilli: appear red bacilli against blue background.



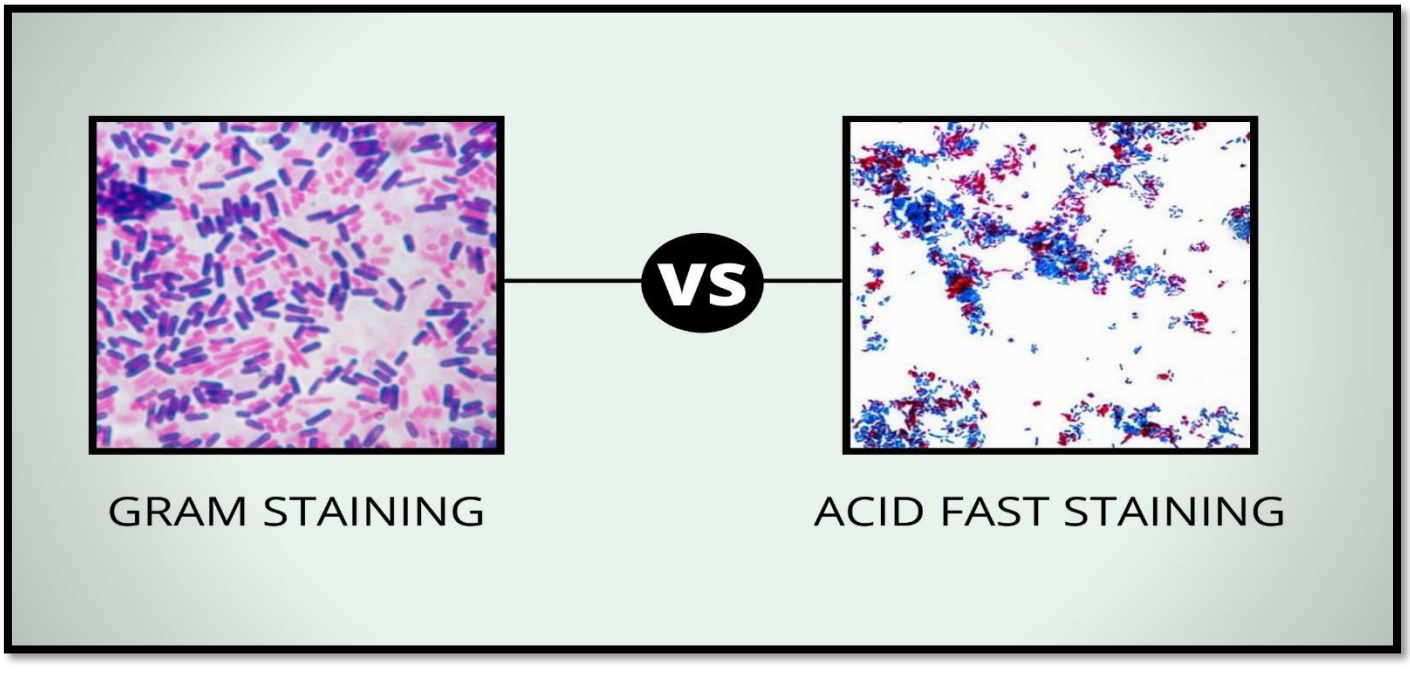


Ziehl neelsen stained smear of acid fast bacilli

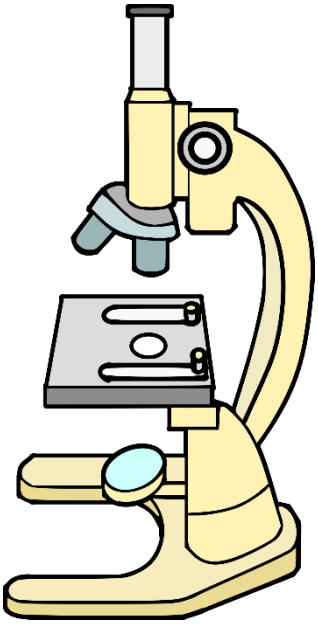
Modified Ziehl-Neelsen's stain

☹ Different concentrations of **Decolorizing agents** were used.

M. leprae	5 % H_2SO_4 and 95% alcohol or 1% HCL in 95% alcohol
Bacterial spores	0.25-0.5 % H_2SO_4
Actinomycetes Nocardia	0.5-1 % H_2SO_4



Special staining



- It is used to color **specific parts** of micro-organism.
- **Examples**:
  1. Capsule stain
  2. Spore stain
  3. Flagellar stain
  4. Silver impregnation stain
  5. Volutin granules stain.

1- Capsule stain

A-Gram stain

☹ Capsule appears as **unstained areas** around the organism.

B- Negative staining

☹ The background is dark grey, the organisms are unstained and the **capsule stands out as a shiny ring** around the organism.

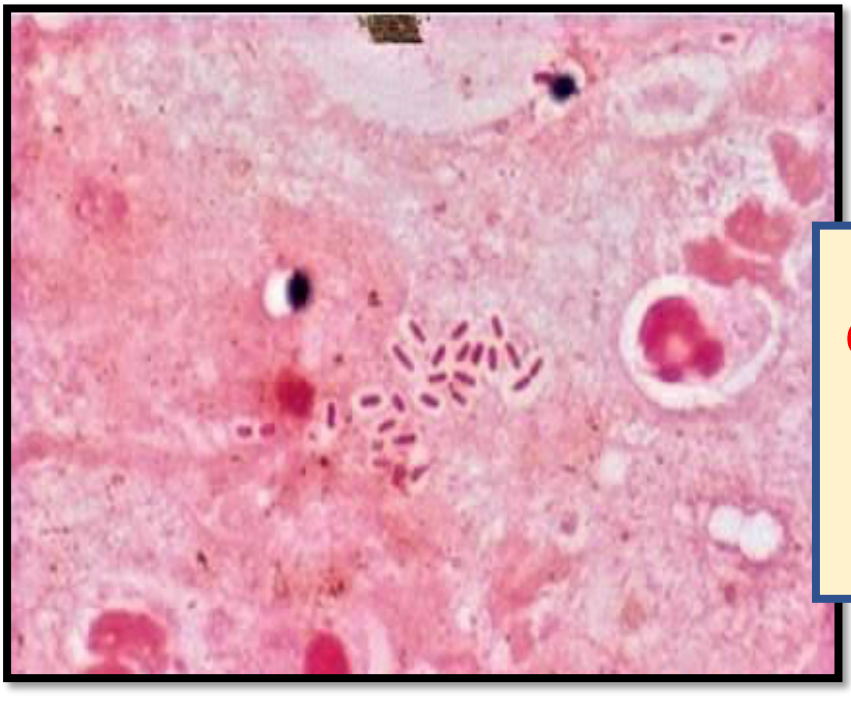
2- Spore stain

A-Gram stain

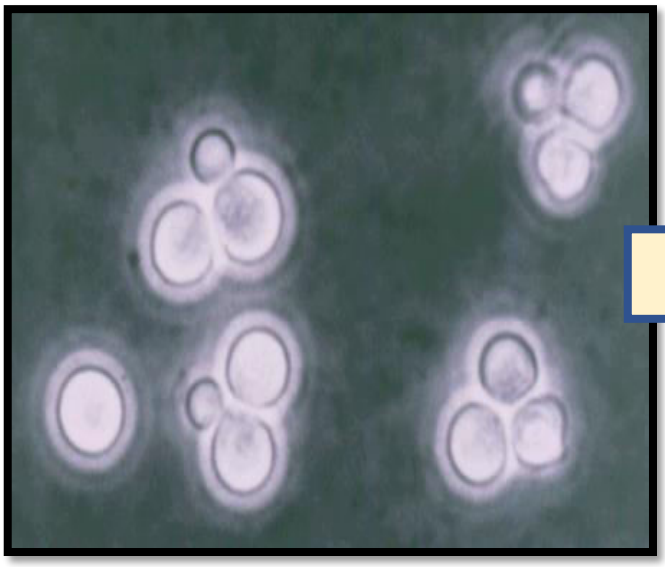
Spores appear as **unstained areas** within a vegetative cell.

B- Modified Z.N stain

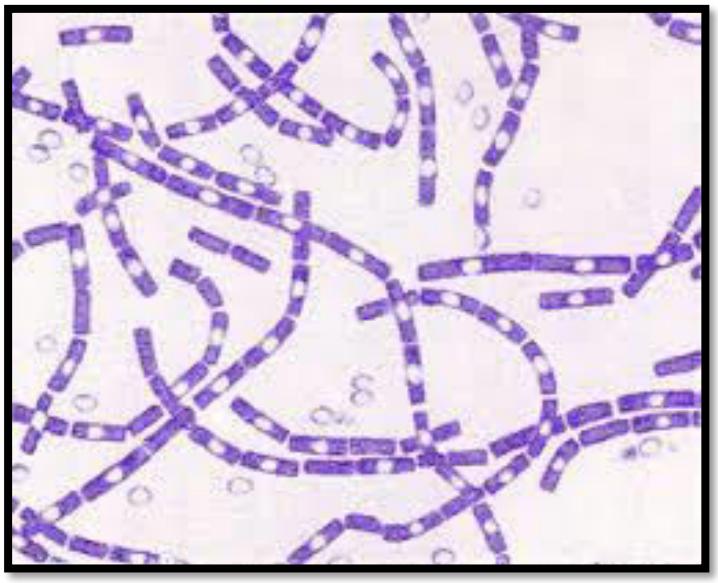
Spores **appear red** and vegetative cells blue.



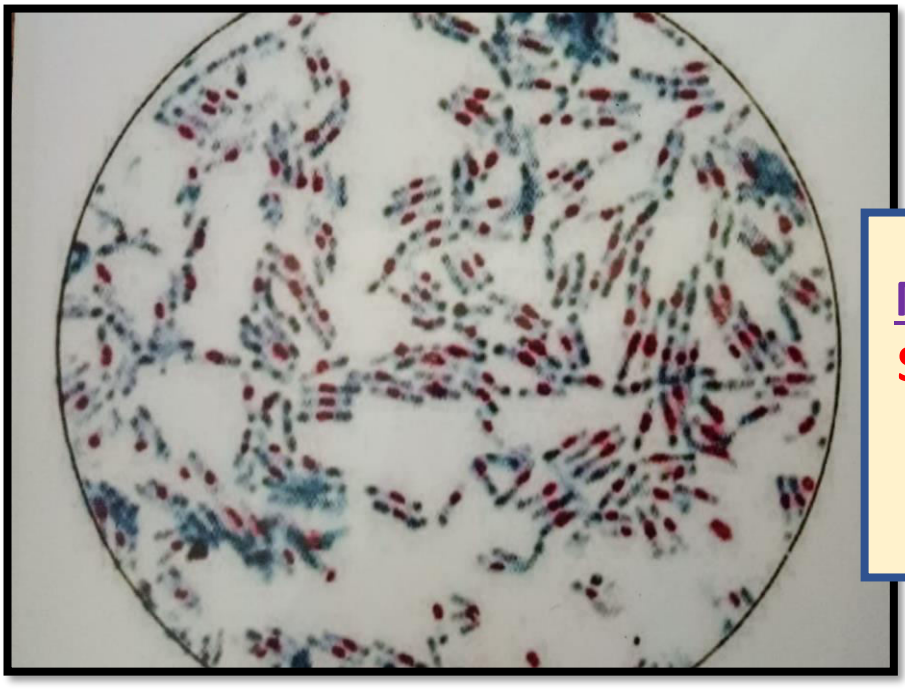
Gram stain:
Capsule appear as unstained areas around the organism



Negative stain



Gram stain: spores appear as unstained areas within a vegetative cell



Modified Z.N stain

Spores appear red and vegetative cells blue

III-CULTURE MEDIA AND CULTIVATION OF MICRO-ORGANISMS

- **Cultivation of bacteria** is a process in which artificial culture media with nutritive components are used to allow bacterial growth in vitro.

- **Bacteria are grown in laboratory to isolate them in pure form in order to:**
  a) To study their properties.
  c) To isolate them from pathological specimens.
  d) To prepare antigens, toxins, vaccines or other products.

Classification of Culture Media

1- According to presence or absence of oxygen

1- Aerobic cultivation.
2- Anaerobic cultivation

2- According to the consistency

A. Fluid media (no agar).
B. Solid media (1.5-2% agar).
C. Semi solid media (0.2-0.5% agar).

3- According to chemical structure

I-Simple Media.
II- Enriched Media.
III- Differential Media.
IV- Selective Media.
V- Sugar Media.
VI- Anaerobic Cultivation.
VII- Blood Culture Media.

I- Simple Media

Contain the essential growth requirements.

	1-Peptone water	2-Nutrient broth	3- Nutrient agar
Type	**Simple fluid media**		**Simple solid medium**
Content	1%Peptone + 0.5% NaCl	Peptone water + meat extract	Nutrient broth+2% agar agar **(solidifying agent).**
Uses	Base for sugar media & indole test.	**Support growth of many microorganisms. Base for other solid media**	
			**Slope agar:** Preservation of identified strains.  **Deep agar:** Anaerobic cultivation.  **Plate agar:** Support growth of many microorganisms Detect pigment production. Biochemical characteristics 

Deep soft agar (0.5% agar gar)

- Used to detect motility of bacteria
- Inoculated by stabbing using **bacteriologic needle.**

II- Enriched Media

Contain highly nutritive substances.

	1-Blood agar	2-Chocolate agar	3- Loffler's serum
Type	**Enriched & Differential**	**Enriched**	
Contents	Nutrient agar + 10% blood (**horse, sheep or human**) **Sterile blood** is added to sterile nutrient agar.	As blood agar then the temperature of medium is raised 100°C for 10 min. to rupture red blood cells and release the nutrients.	3 parts sterile serum (**horse, sheep**) + 1 part glucose broth.
Steriliz.	Nutrient agar by autoclave + Sterile blood added to it at 55°C	As blood agar	Inspissations (1-2 hours at 80°C on 2 successive days)
Uses	**Growth of fastidious bacteria** as *sterptococci.* **Differentiate between bacteria** according to type of haemolysis (α, β, γ).	**Growth of fastidious bacteria** as *Neisseria* and *Haemophilus.*	**Growth of** *C. diphtheria.*

III- Indicator media

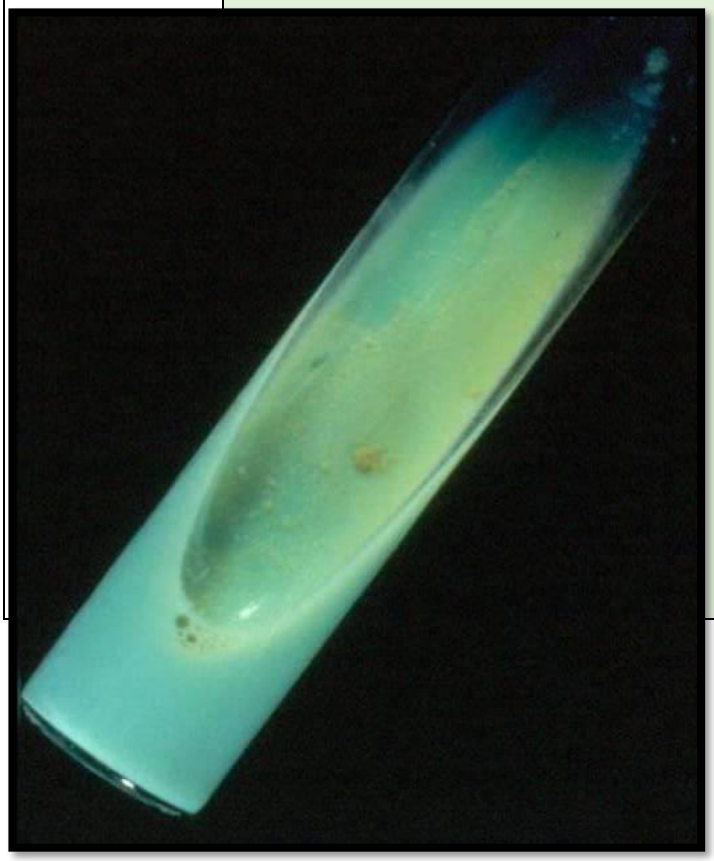
They contain an indicator that changes its color according to pH due to metabolic activities of particular organisms.

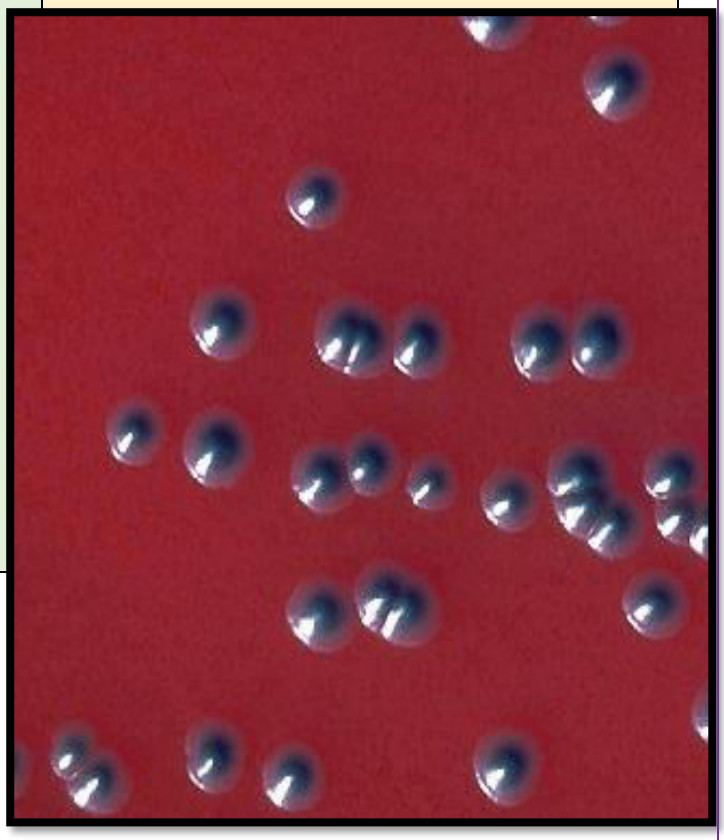
	1-MacConkey's agar	2-Tripple sugar iron agar
Type	Differential, indicator & Selective	Indicator
Contents:		
Nutrient	Peptone	Beef & yeas extract
Sugar	Lactose	0.1% glucose, 1% lactose, 1% sucrose
Indicator	Neutral red	Phenol red/ Ferrous sulfate
Selective	Bile salts	-----
Uses	Isolation of Enterobacteriaceae & differentiate between lactose fermenter & lactose non fermenters	Differentiation of Enterobacteriaceae by testing their fermentative activity and H2S production

IV-Selective Media

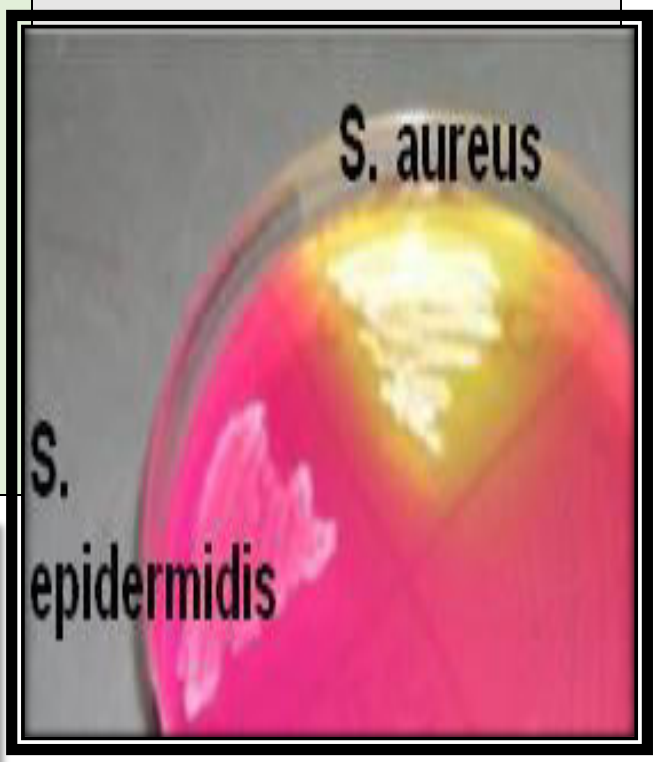
They contain inhibitory substances that inhibit growth of certain bacteria and allow growth of others

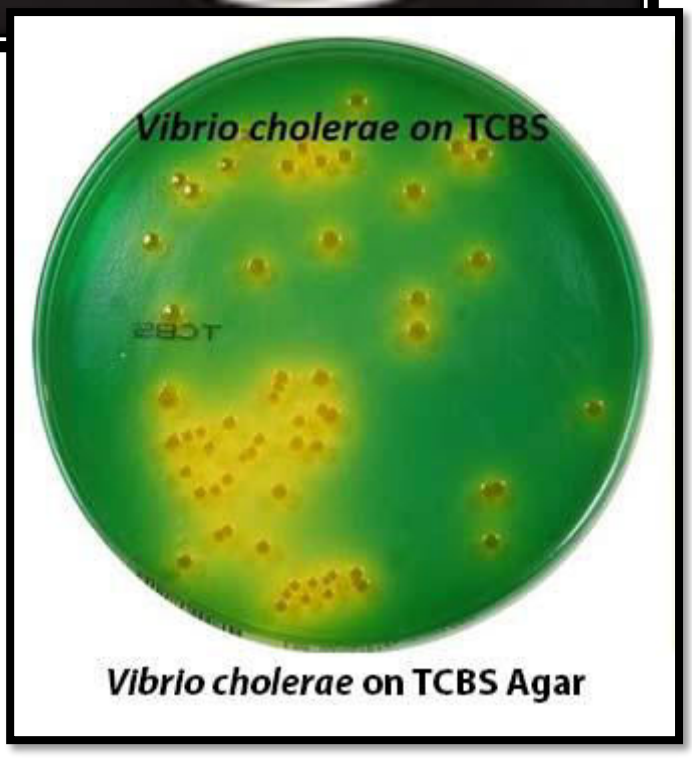
	1) Lowenstien Jensen media (LJ)	2) Blood tellurite
Type	Selective, enriched	Selective, enriched & differential
Nutrition	Whole eggs	Blood
Selective substance	Malachite green	Potassium tellurite
Sterilization	Inspissation	As blood agar
Uses	Selective for *Mycobacterium tuberculosis*	Selective for *C.diphtheria*, Differentiation of types of *C.diphtheria*





	3)Thiosulphate-Citrate-Bile salt Sucrose (T.C.B.S)	4) Mannitol salt agar
Type	Selective /indicator	Selective /indicator
Selective substance	Na thiosulphate, Na citrate and Bile salt. +Alk PH	7.5% NaCl.
Indicator	Sucrose and bromothymol blue	Mannitol/Phenol red
Uses	Selective for isolation of *Vibrio*	Selective for isolation of *Staphylococcus aureus*.





V-Enrichment media

Contain substances that allow the growth of certain organisms and not others.

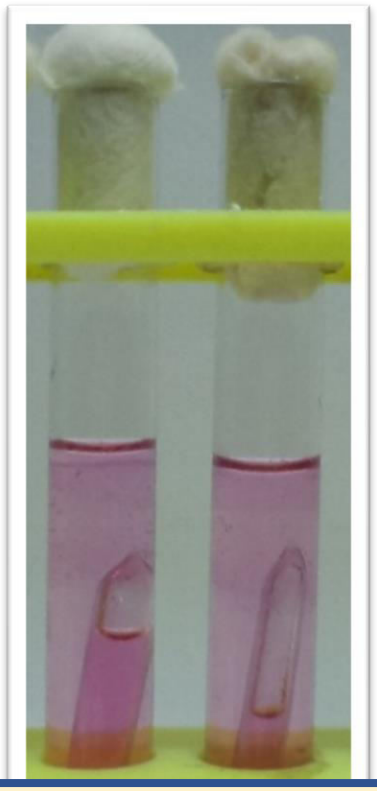
1) Selenite broth	2) Tetrathionate broth	3) Alkaline peptone water
Enrichment liquid medium	Enrichment liquid medium	Enrichment liquid medium
Peptone water Na selenite	Nutrient broth Tetrathionate solution	Peptone water Alkaline pH (8-9)
Enrichment media for *Shigella* in stool.	**Enrichment media for** *Salmonella* in stool.	**Enrichment media for** *V. cholera in stool.*

VI- Sugar Media

- **Bacteria vary** in their fermentative action on different sugars.
- Bacteria ferment sugar and produce **acid alone** or **acid and gas**.
- **Each test tube contains**: 1% **sugar**, in **peptone water**, **Andrade's indicator** and **Durham's tube.**
- **Initially**, the color of the medium is **colorless** or **light yellow** and if organism ferment sugar, acid is produced which is indicated by change of color to **pink**.
- **Durham's tube** is kept inverted to detect **gas** production.
- **Sugars are**: glucose, lactose, maltose, mannite, sucrose.

All media are sterilized by autoclave except:

1. **Loffler's serum & Lowenstien Jensen** are sterilized by inspissation (heating for 1- 2 hr. at 80°C)
2. **Blood agar**: sterile blood is added to sterile nutrient agar (autoclave) its temp. reaches 55° C.
3. **Sugar media** are sterilized by tyndalization or filtration.



Fermentation with production of acid and Gas

Fermentation with production of acid only

VII- Anaerobic cultivation

Some bacteria cannot grow in the presence of oxygen so anaerobic condition is essential for their isolation.

Anaerobiosis can be achieved by:

1-Anaerobic culture media:

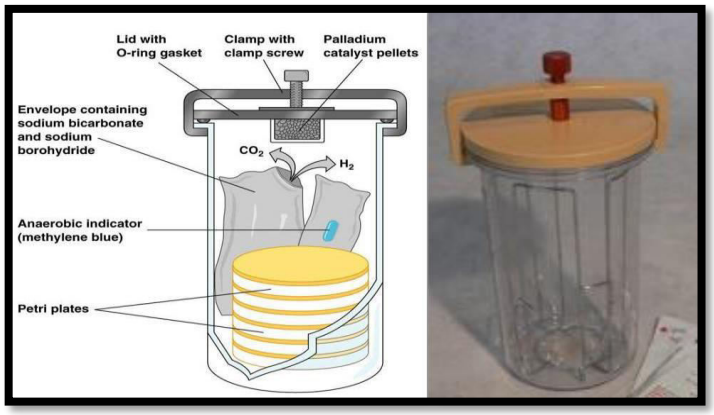
- ***Robertsons's cooked meat broth*** and ***Thyoglycolate broth***.
- These media contain reducing substances to reduce oxygen and provide anaerobic condition.
  Thyoglycolate broth is also used as transport medium.

2-Anaerobic GasPack system:

- It is especially tightly fitted lid jar.
- Anaerobic condition is achieved by releasing hydrogen from commercial envelope to combine with oxygen in the presence of catalyst.

3-Non –gas generating system- oxygen removing system:

- An oxygen removing chemical (**ascorbic acid**) is placed in simple jar (does not produce hydrogen and does not need catalyst).

		
Robertsons cooked meat	**Anaerobic GasPack system**	**Simple jar**

IV- BIOCHEMICAL REACTIONS & IDENTIFICATION OF THE BACTERIA

It is essential to start identification tests with pure bacterial isolates grown from single colony.

A) Conventional methods

1-Colonial Morphology
2-Microscopic examination from colonies
3-Biochemical reactions
4-Animal Pathogenicity
5-Serological (immunological)
6-Phage typing

B) Non-conventional

1-Monoclonal antibody
2-Flow cytometry
3-Molecular biology

BIOCHEMICAL REACTIONS

Metabolism of CHO
-Action on sugars
-Methyl red test
-Voges -Proskaur's test

Action on proteins
- Indole
- H2S production
- Gelatin liquefaction

Combined
Litmus milk

Enzyme production
-Coagulase
-Catalase
-DNase
-Oxidase
- Urease

Ability to utilize specific substrate
- Citrate utilization test

A) Metabolism of CHO

1-Sugar fermentation

Principle: Ability of bacteria to ferment sugars (with acid production ± gas).

Interpretation:

- **Acid** is detected by Andrade's indicator that changed into **red** color.
- **Gas formation** is detected by collection of **bubbles** at apex of inverted Durham's tube.

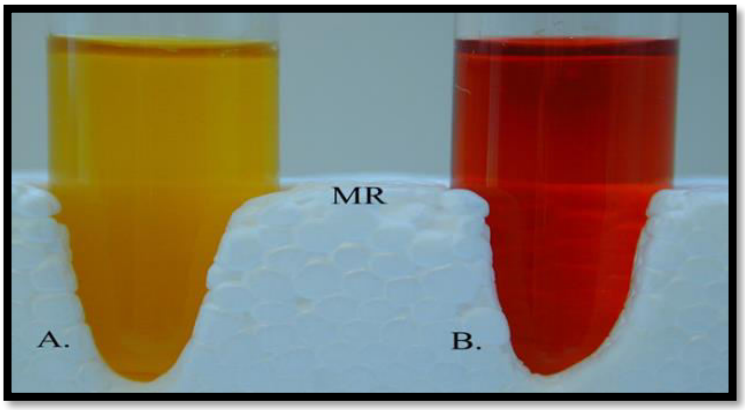
2) Methyl red (MR) Test

Principle: this test detects the production of sufficient acid during fermentation of glucose by bacteria and lowering of pH below 4.

Interpretation

Positive: **Red** color

Negative: **Yellow** color



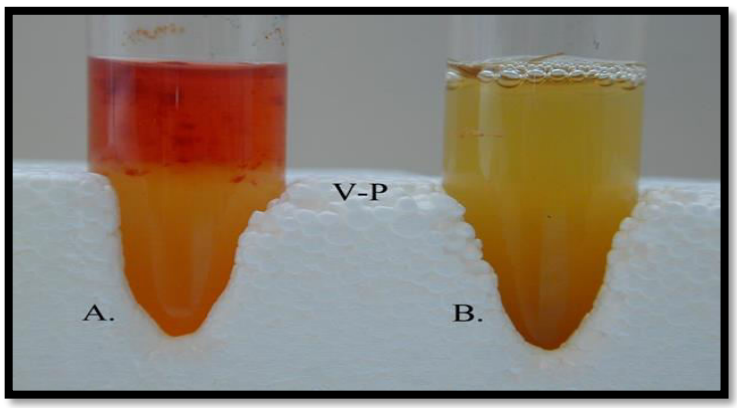
3)Voges- Proskauer (VP) Test

Principle: It depends on the production of acetyl methyl carbinol from pyruvic acid in the media.

Interpretation:

Positive: **pink** color

Negative: Colorless or yellow**.**



N.B: A culture will usually only be positive for one pathway: either MR+ or VP+.

- ***Escherichia coli*:** MR+ and VP-.
- ***Klebsiella pneumoniae**:* MR- and VP+.
- ***Pseudomonas aeruginosa**:* a glucose non-fermenter and is thus MR- and VP

B) Action on proteins

1-Production of indole

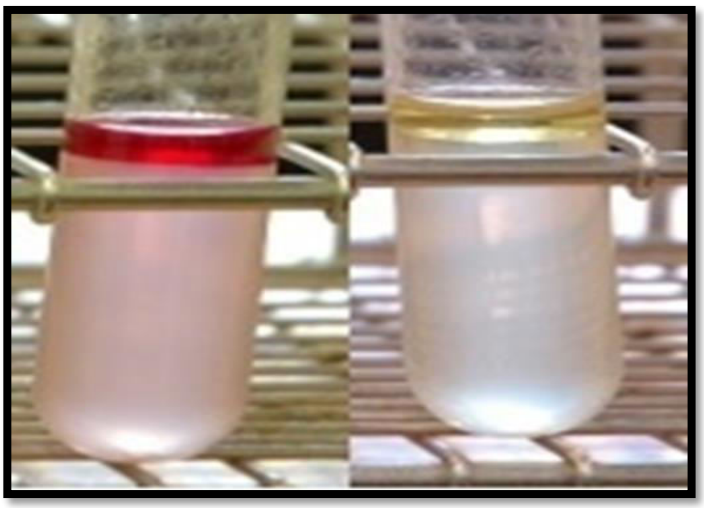
Principle: Indole production from amino acid tryptophan.

Media used: Peptone water

Interpretation:

Positive: **Pink** ring

Negative: **Yellow** ring

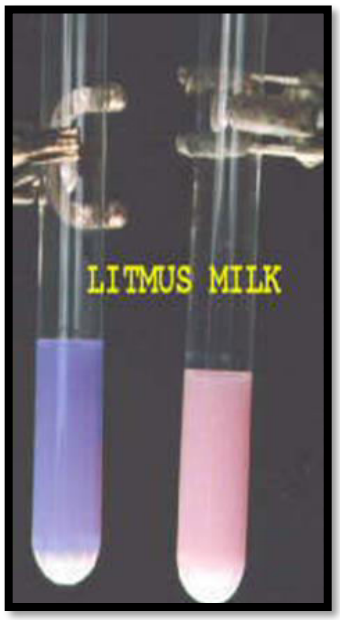


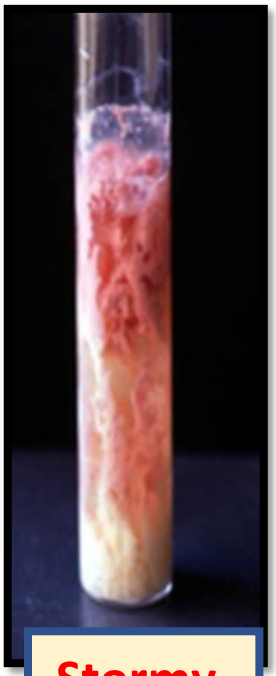
2-H2S production

Principle: Depends on production H2S from sulfur containing amino acids.

Media used: peptone water

Interpretation: *Positive test* ⟶ ***blackening***





Stormy clot

C- Combined action on CHO &proteins

Litmus milk

Principle: Ability of some bacteria to ferment lactose in milk and clotting of milk due to acid production.

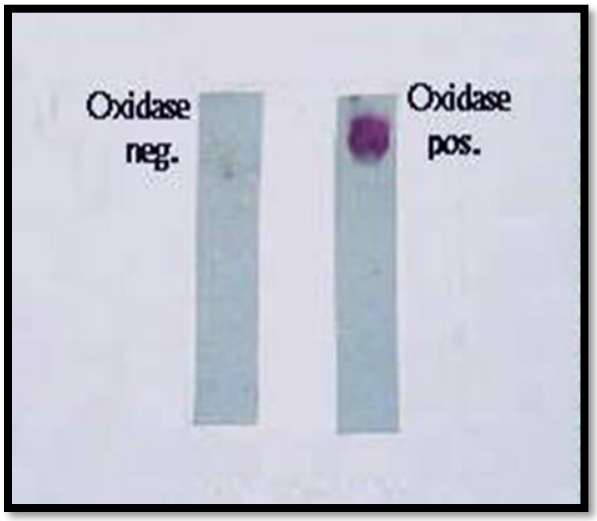
Media used: Skimmed milk +litmus indicator

Interpretation:

- Certain bacteria cause acidity⟶ pink
- Clostridium perfringens → stormy clot (large amount of acid and gas)
- Certain bacteria cause alkalinity ⟶ blue

D)Enzyme production

1-Oxidase test



Principle: Some bacteria produce cytochrome oxidase enzyme (Neisseria,Vibrios and Pseudomonas).

Interpretation:

Positive test: Immediate purple color (10 sec.)

Negative test: No purple color develops.

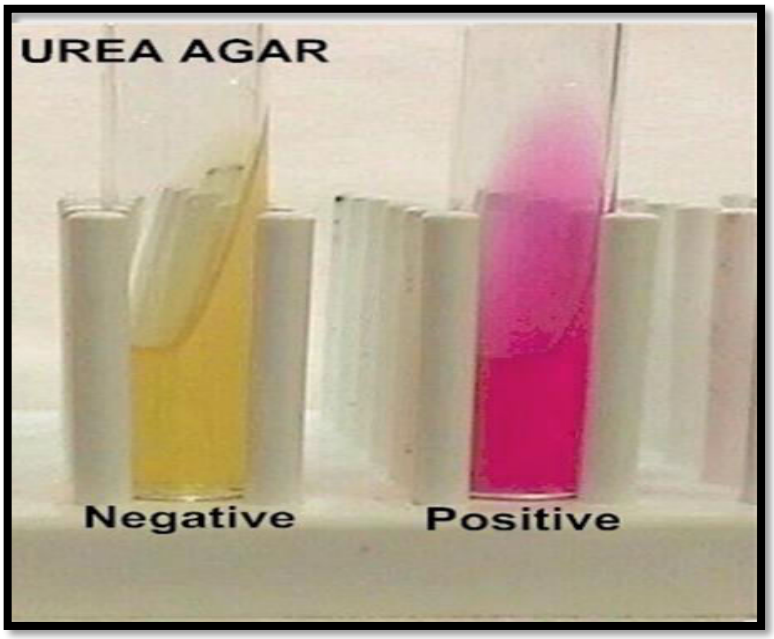
2-Urease test

Principle: Some bacteria produce urease enzyme that split urea to ammonia and CO2. Ammonia causes alkalinity of medium that is detected by **phenol red indicator**.

Interpretation:

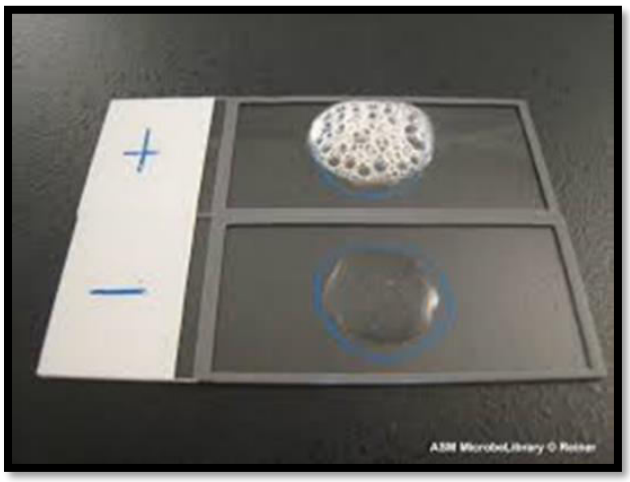
Positive test: Deep pink after 4-24h (proteus).

Negative test: Yellow



Urease test

3-Catalase test



Principle: Some bacteria produce catalase enzyme

Interpretations:

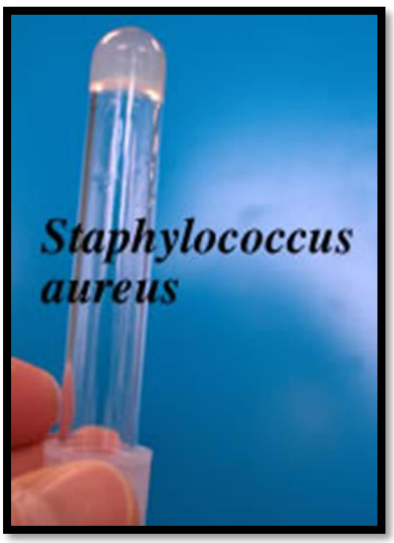
Bubbles = Positive

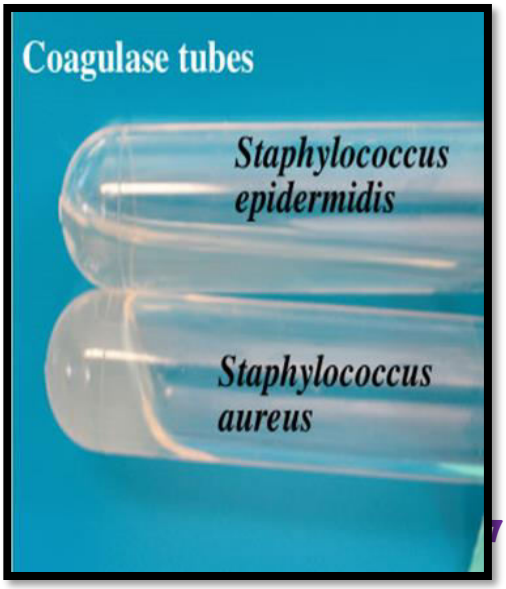
no bubbles = negative

4-Coagulase test

Principle: Some bacteria produce coagulase enzyme (free, bound)

- Fibrinogen (plasma) ⟶ fibrin (clot)
- Bound coagulase (clumping factor) directly conversion. Detected by rapid **slide method**
- Free coagulase needs activation of coagulase-reacting factor in plasma detected by **tube method**





5-DNase production test

Principle: Some bacteria produce DNase enzyme that hydrolyze DNA into smaller molecules.

Interpretation:

Negative: opaque white

Positive colonies surrounded by clear zones

E-Ability to utilize specific substrate

Citrate Utilization Test:

Principle: It is the ability of an organism to utilize citrate as the sole source of carbon for its growth.

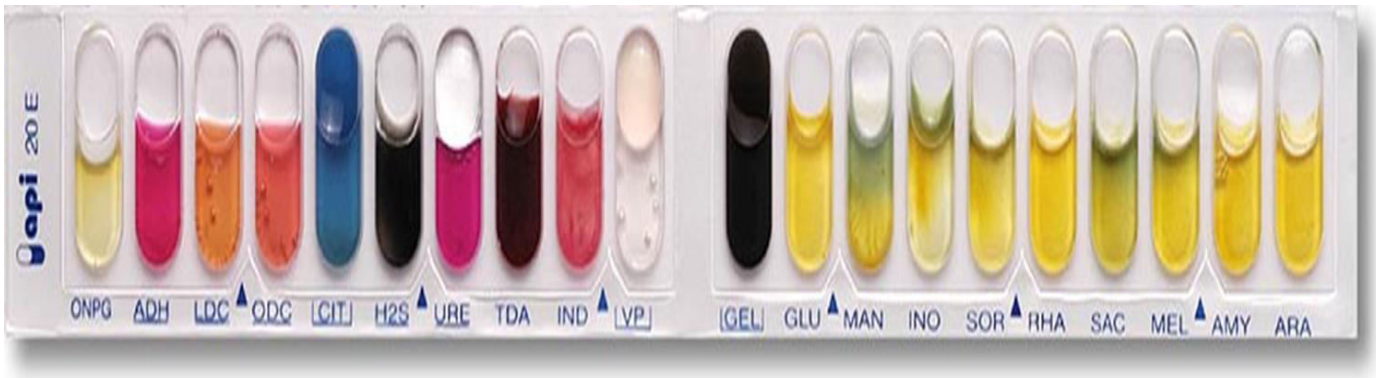
Interpretation:

Positive: Growth with an (**intense blue**) color on the slant.

Negative: No growth with any change in color (**green**).

Analytic Profile index (API)

- A Commercial kit system for bacterial identification.
- It is a plastic strip with mini cupules and tubes, each with a reagent test for different bacterial metabolism.
- Interpretation of positive and negative tests depending on color change which is converted to numerical code that allow identification of organism



Automated Systems

- Microbiology laboratories are using it to identify bacterial pathogens (Vitek System, Micro scan, Phoenix).
- This method determines the metabolic activity of the organism for its identification.

Serological tests

- Detection of microbial antigens by using antibodies for serotyping of organism.

Animal pathogenicity test

- Animals commonly used are guinea pigs, rabbits, mice.
- **Importance:**
  - Differentiate pathogenic and nonpathogenic.
  - To test ability of toxin production.

Bacteriophage typing

Used for:

1- Bacterial identification (to subspecies or strain level)
2- Typing (determining strain) in outbreaks.

II- Non conventional methods

A- Monoclonal antibodies
B- Flow cytometry
C-Molecular biology methods

A-Monoclonal antibodies

- For Direct detection of bacterial antigen in specimens

B-Molecular biology methods

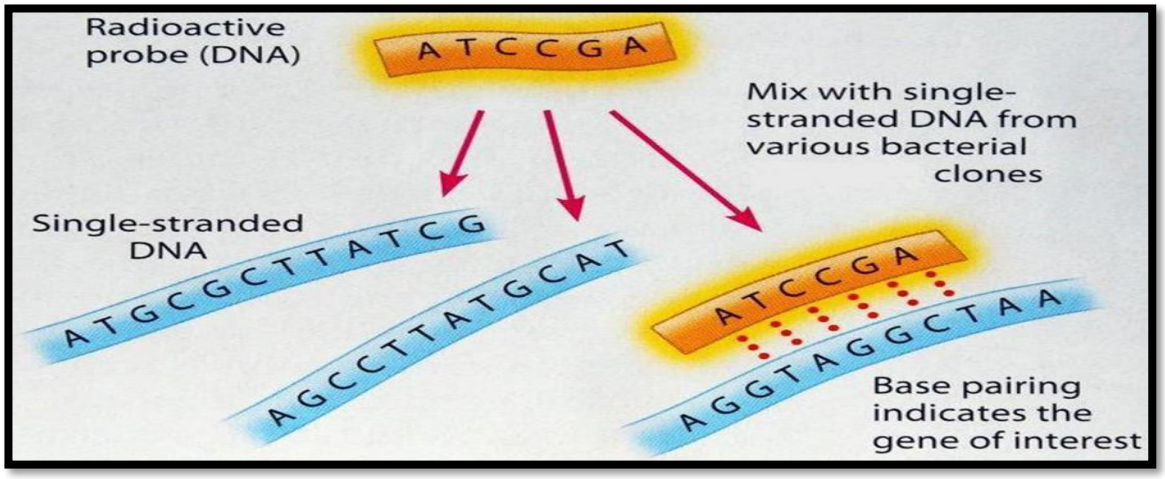
Each bacterial species contains specific **DNA** or **RNA** that contain **unique sequence of nucleotides** that can be used for its identification**.**

Molecular methods include

- **Nucleic acid probes.**
- **Polymerase chain reaction (PCR).**
- **Restriction endonuclease analysis.**
- **Plasmid profile analysis.**

1- Nucleic acid probes

- ✓ short sequences of labeled single stranded DNA or RNA by radioactive-isotope or enzyme.
- ✓ The labeled probe is used to detect the presence of complementary sequence in microbial nucleic acid in clinical specimens or isolated cultures.

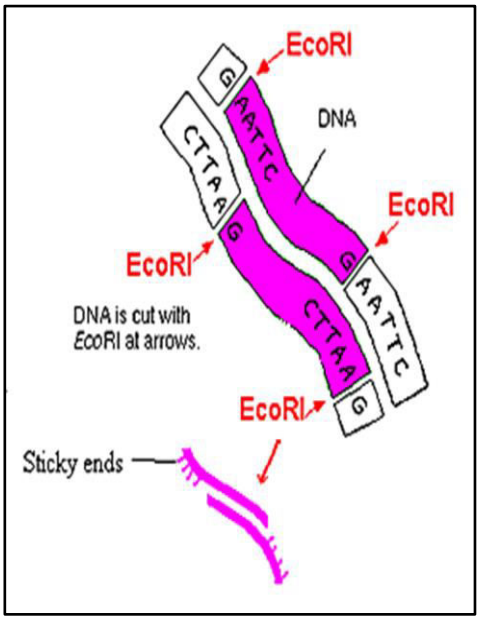


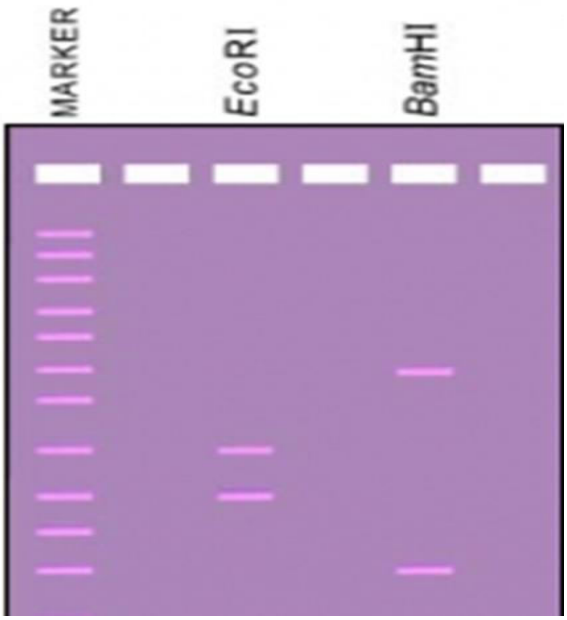
2-Polymerase Chain Reaction (PCR):

- ➢ If the organism is present in small number that cannot be detected by probe so amplification of target DNA or RNA leading to accumulation of several copies (billions) of that short sequence that can be detected easily.

3-Restriction Endonuclease Analysis:

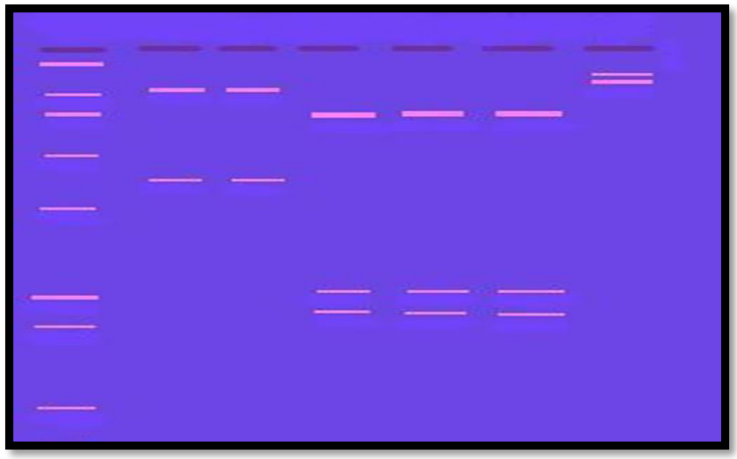
- ❖ Restriction endonuclease (RE) is a class of bacterial enzymes that cut DNA at specific sequence sites.
- ❖ Differences in the nucleotide sequence of the chromosome result in generation of fragments of variable lengths when digested with (RE).
- ❖ The resulting restriction fragments are separated by electrophoresis on agarose gel





4-Plasmid Profile Analysis:

- Similar bacterial strains often carry similar types and numbers of plasmids.
- Plasmid DNA is isolated from the bacterial cells, and then separated on agarose.
- The presence of identical plasmid pattern in a group of bacterial isolates indicates that they are **related.**



V- ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST) (ANTIBIOGRAM)

It is an in-vitro laboratory method which determines the susceptibility of bacteria to antimicrobials.

Uses of AST:

- Direct the clinicians in the selection of antibiotics.
- Control the use of antibiotics in clinical practice.
- Determination of resistance pattern of different bacterial isolates.

Methods of AST

1-Diffusion: Disc diffusion
2-Dilution: - Broth dilution
- Agar dilution.
3- Diffusion and Dilution: Epsilometer (E) test
4-Automated sensitivity test

1-Disc diffusion method (Kirby-Bauer) method

The most commonly used method to determine antibiotic sensitivity.

Method:

1- Make bacterial suspension from isolated organism in sterile saline or broth.
2- Inoculate this suspension on the surface of suitable medium (**Mueller Hinton sensitivity agar**).
3- Place the appropriate antimicrobial discs on the surface of the inoculated plate
4- Examine the plates for the presence of zones of inhibition of bacterial growth around antibiotic disks.

These are measured by ruler from under surface of the plate.

The size of zone of inhibition is dependent on the diffusion rate of the antibiotic, degree of sensitivity of the microorganism, and growth rate of bacterium.

Antibiotic discs

Disc diffusion method

2-Tube dilution method

The use of this quantitative method is limited to the following situations:

1. No response to antibiotic treatment.
2. Relapses during antimicrobial treatment.

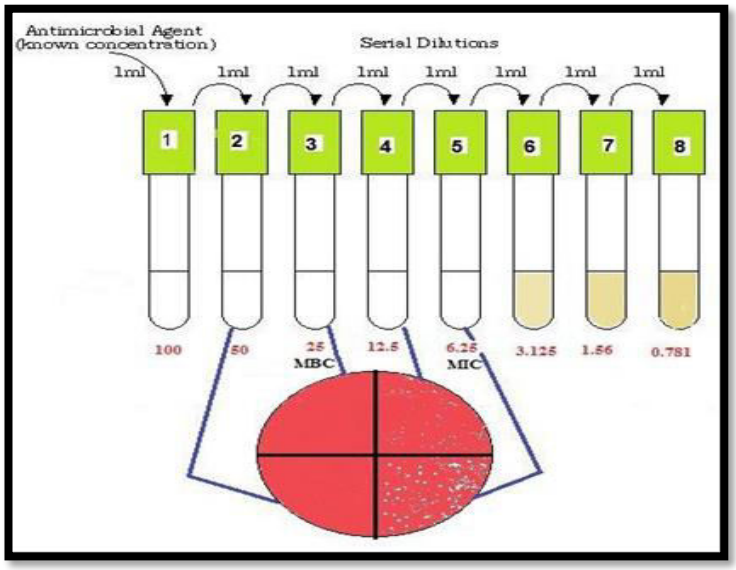
Method:

1. Two folds dilution of the antibiotic is prepared in suitable sterile nutrient fluid medium (nutrient broth).

2. Two control tubes are included in each test, **growth control** (no antibiotic was added) and **sterility control** (no organism was added to these tube).
3. After incubation for 18- 24 hours at 37°C the tubes are examine for turbidity.

- **MIC= Minimal inhibitory concentration**: The tube with highest dilution of antibiotic showing no visible growth.
- **MBC= Minimal bactericidal concentration**: the tube with highest dilution that fail to yield growth on the subculture plate. MBC can be determined by subculturing all tubes showing no visible growth on suitable medium such as blood agar.

Tube dilution method and subculture on blood agar To identify MBC



3-Epsilometer or "E" test

It is a quantitative method for direct determination of the MIC. A gradually increasing concentration of antibiotic is fixed along a rectangular plastic test strip (commercially available) which is applied to the surface of an inoculated agar plate. After incubation a tear drop shaped inhibition zone is seen. The zone end indicates MIC of the antibiotic.

4-Automated sensitivity test

An automated system to identify bacteria and test their sensitivity rapidly.

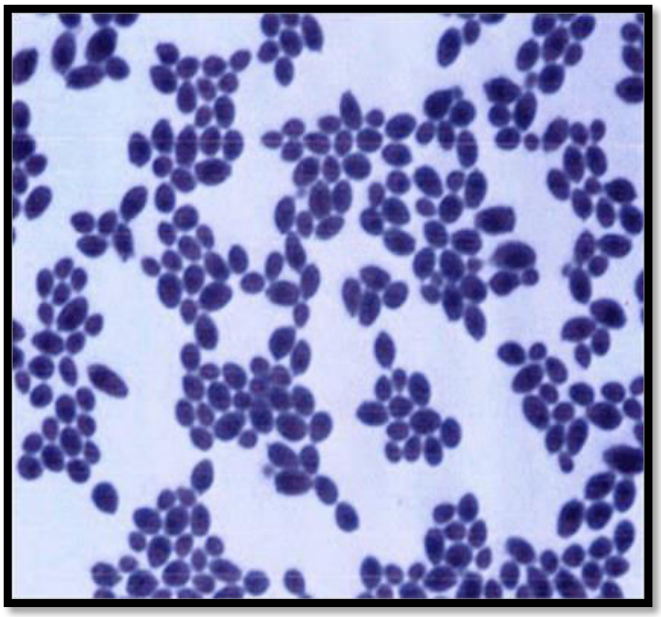
Laboratory diagnosis of fungal infections

1-Specimen collection:

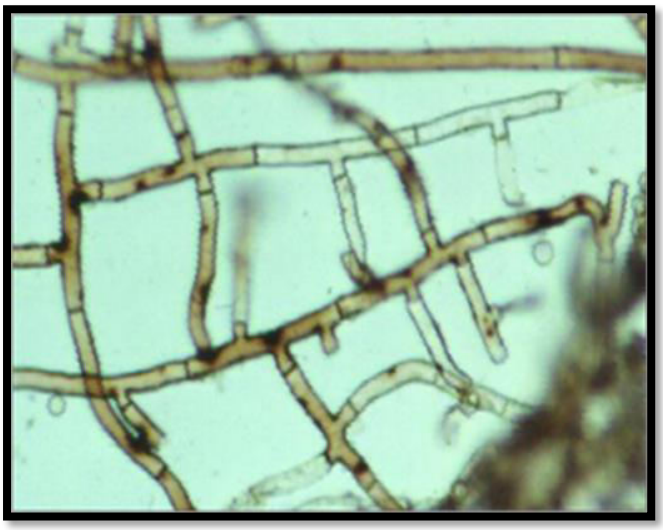
- No special attention is needed.
- Only thick keratinized specimens (skin scrapings, nails, hairs) are dissolved in 5-20% KOH to clear the spores (time depends on amount of keratin).

2-Direct microscopy:

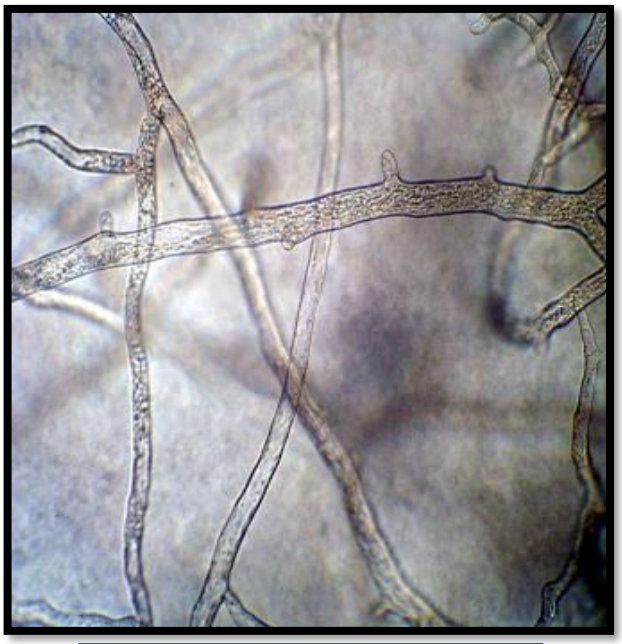
- **The most common stains used are:**
  - ✓ Gram stain.
  - ✓ Lactophenol cotton blue.
  - ✓ **India ink**: for capsulated fungi.



Gram positive
Yeast cells
Candida albicans



Septate hyphae



Aseptate hyphae

3-Culture:

- **Sabouraud's dextrose agar (SDA):** for molds.
  SDA contains **Chloramphenicol** (inhibits bacterial growth) and **cycloheximide** (inhibits growth of saprophytic fungi).

- **Brain-heart infusion agar**: for yeasts

- **Incubation temperature**: 37°C for yeasts, room temperature for molds
- **For dimorphic fungi**: one plate at room temperature and another plate at 37°C.
- **Macroscopic examination** of the mold colony is also very essential.

SDA

Candida on SDA

Aspergillus niger

Aspergillus flavus

4)Biochemical reactions:

- **Sugar fermentation**
- **Sugar assimilation:** ability of fungi to use certain sugar as the only source of carbon.
- **Germ tube formation**: is done to confirm the diagnosis of **Candida albicans**.
- **Chlamydospores formation:** **C. albicans** form true mycelia and round terminal chlamydospores when cultured on corn-meal agar.
- **Urease test** → Cryptococcus neoformans.

5) Serological tests: may be useful in deep fungal diseases.

6) Skin tests.

7) Molecular techniques: DNA probe or PCR