



Laboratory Diagnosis of Bacteria

Lab/ 1

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Introduction :

- Bacterial identification is a crucial process in microbiology, essential for diagnosing infections, determining appropriate treatments, and tracing the sources of outbreaks.
- Bacterial identification involves several methods, each with its own strengths and applications.
- These methods can be used individually or in combination to achieve accurate and reliable bacterial identification.

Types of samples:

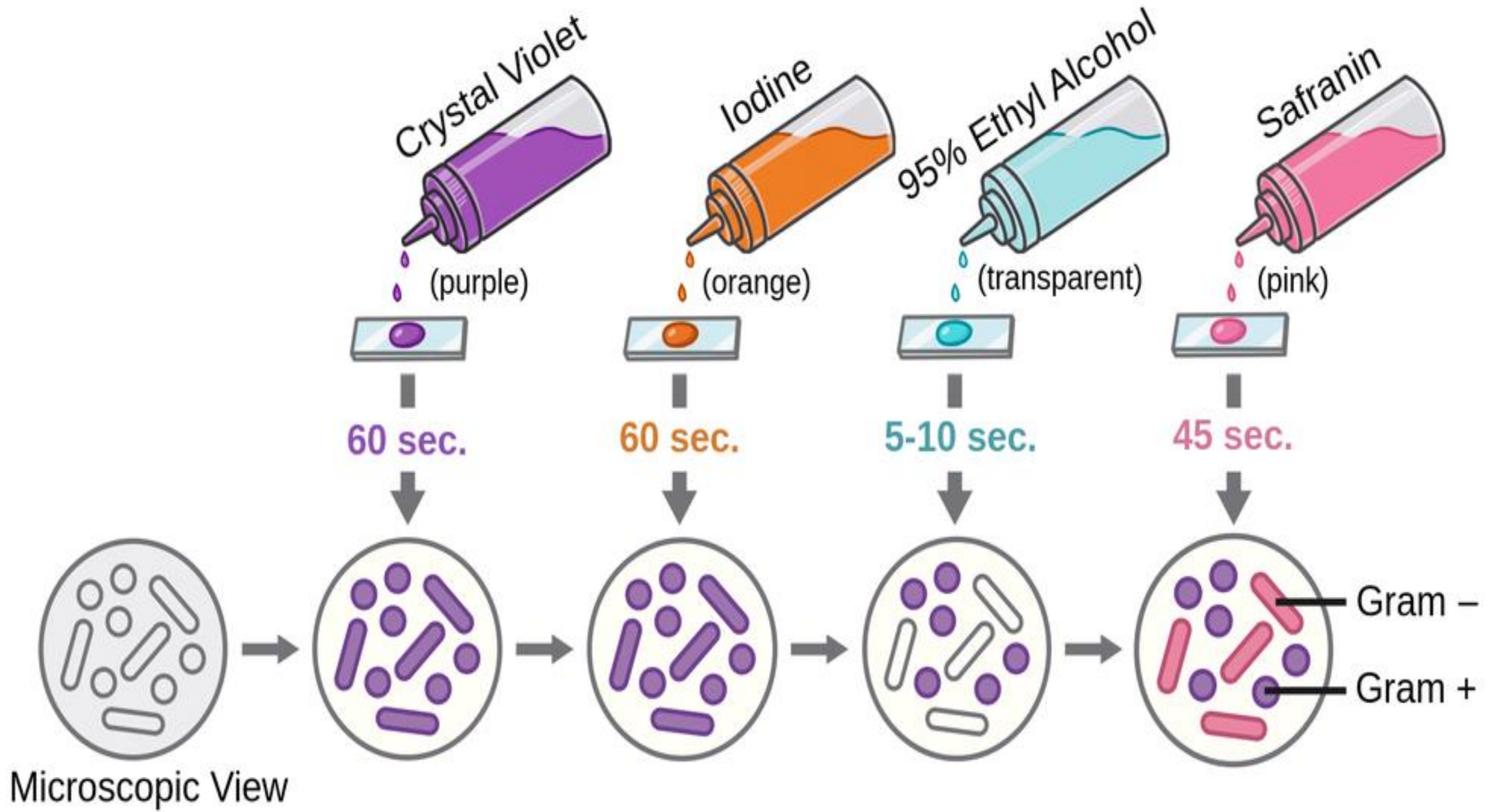
- Blood
- Swab
- Sputum
- Urine
- Feces
- Other body fluids: Spinal fluid, Synovial fluid, Pericardial and Peritoneal fluids

Method of bacterial identification:

- Microscopic examination
- Macroscopic examination (Cultural appearance)
- Biochemical reactions
- Antibiotic susceptibility testing (AST)
- Serological identification
- Molecular method

Microscopic examination

- ❑ **Gram staining:** is a widely used technique in microbiology for the microscopic examination and differentiation of bacterial species ,where bacteria are characterized by structural differences in their cell walls. Bacteria are classified into two groups:
 - Gram-positive bacteria: Have a thick peptidoglycan layer that retains the crystal violet stain, appearing purple under the microscope.
 - Gram-negative bacteria: Have a thinner peptidoglycan layer and an outer membrane, which does not retain the crystal violet stain but takes up the counterstain (safranin), appearing pink or red.



Microscopic View

Macroscopic examination

Macroscopic examination of bacterial cultures involves observing the visible characteristics of bacterial colonies grown on solid media. This examination provides valuable initial information about the type of bacteria present. Here are some key aspects:

1- Color (pigment production):

- Endopigment (restricted to the colonies): for example golden yellow with *Staphylococcus aureus*
- Exopigment (the color diffuses in the surrounding medium): for example green exopigment with *Pseudomonas aeruginosa*



Endopigment

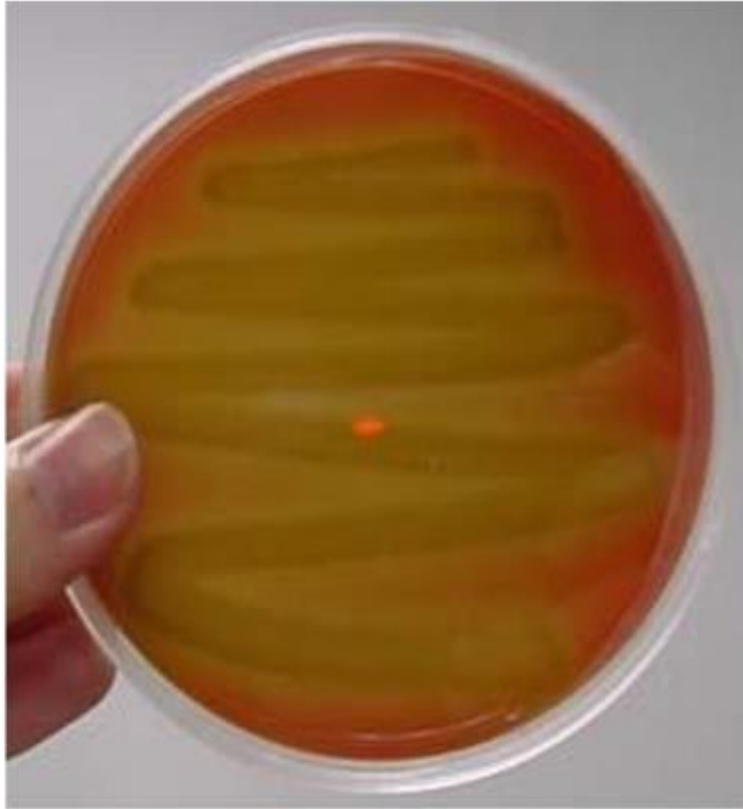


Exopigment

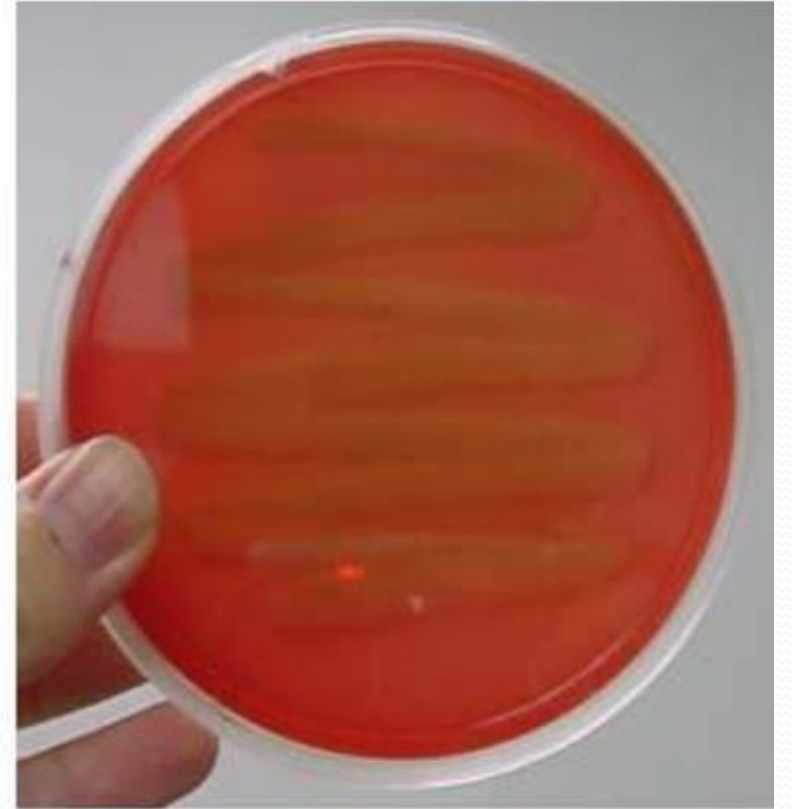
Hemolysis on blood agar:



Beta Hemolysis



Alpha Hemolysis



Gamma Hemolysis

Effect of lactose on MacConkey agar:

- **Lactose fermenters :**

- Appear as pink colonies
- Example : *E.coli* & *Klebsiella*

- **Non lactose fermenters :**

- Appear as pale colonies
- Example: *Salmonella* & *Shigella*



Macroscopic examination

- 2- Colony Shape: Circular, irregular, filamentous, or rhizoid.
- 3- Size: Measured in millimeters, indicating the diameter of the colony.
- 4- Texture: Smooth, rough, mucoid, or dry.
- 5- Elevation: Flat, raised, convex.
- 6- Growth Patterns in Broth: Turbidity (cloudiness), pellicle (surface growth), sediment (bottom growth), or flocculent (clumpy).

Biochemical reactions

- Urease test
- Oxidase test
- Catalase test
- Coagulase test
- DNase test
- Indol test
- Gelatinase test
- Sugar fermentation

Catalase test:

- Is used to differentiate between Staphylococci (catalase +Ve) and Streptococci (catalase -Ve)

- Procedure

-smear a colony of the organism to a slide

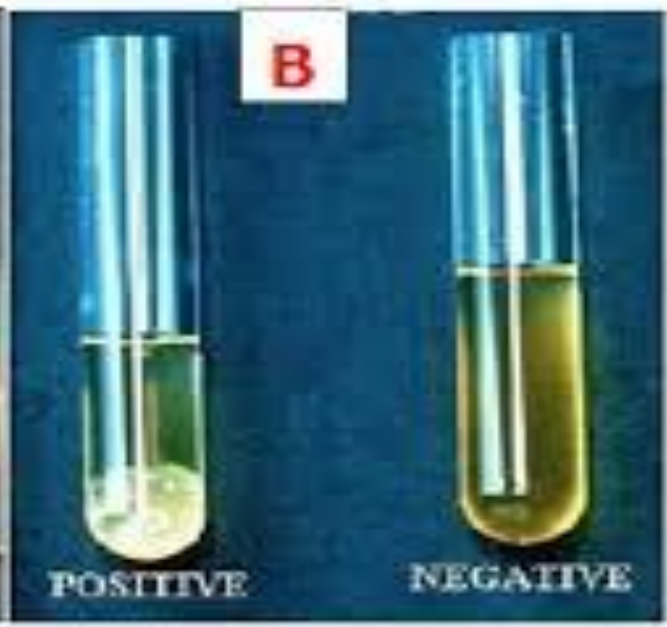
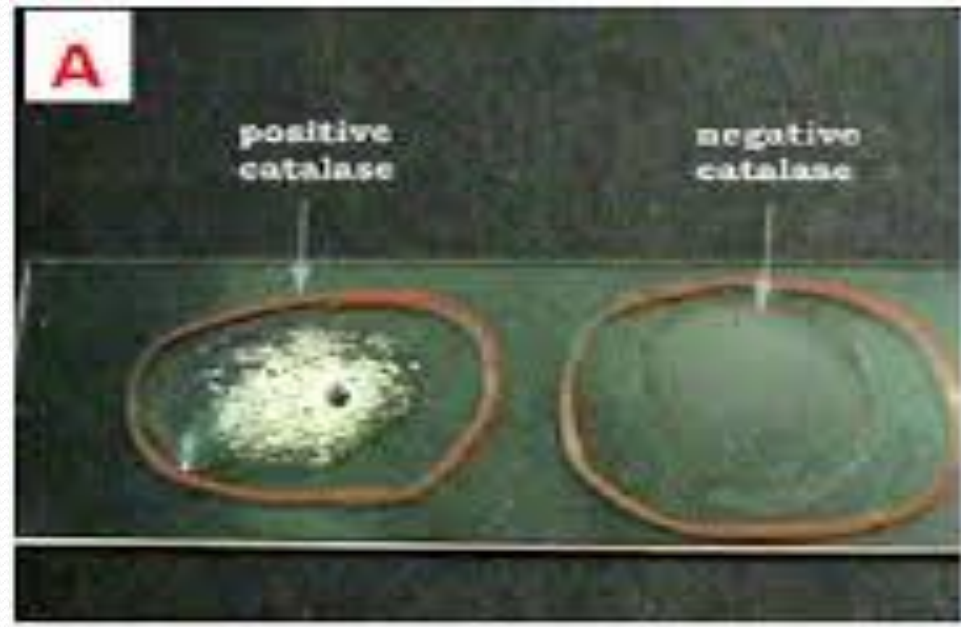
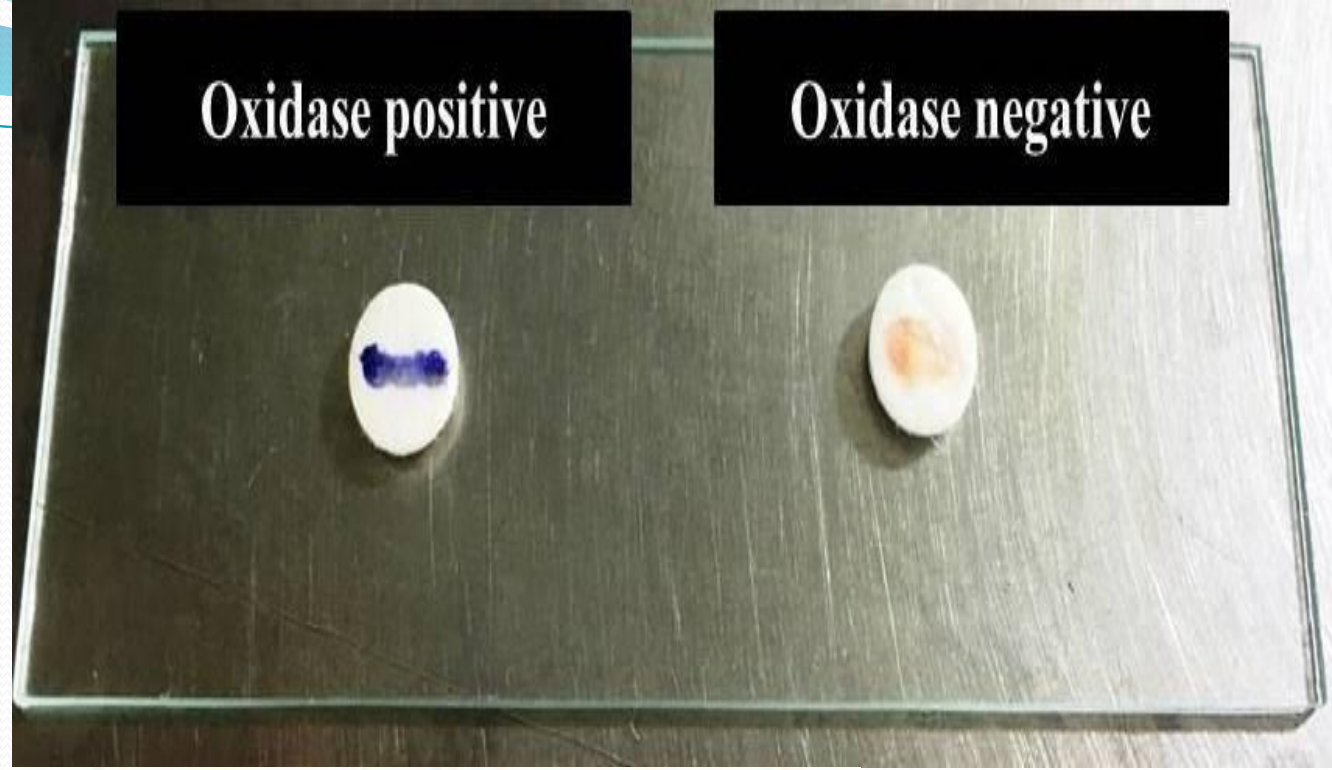
Drop H₂O₂ on to smear

Coagulase test:

- Is used to differentiate Staphylococcus aureus from coagulase -Ve Staphylococci

Oxidase test:

- Is used to differentiate between Enterobacteriaceae from Pseudomonas which is oxidase positive



Automated bacterial identification systems :

VITEK 2 system:

- This system identify the organism and it is antibiotic sensitivity by detecting color changes or turbidity in special plastic cards inoculated with the organism
- such cards are composed of tiny wells that contain substrates for detection of biochemical reactions and antibiotic sensitivity
- once the card has been inoculated and placed in the instrument, it will automatically perform all readings
- results are available within 4-6 hours

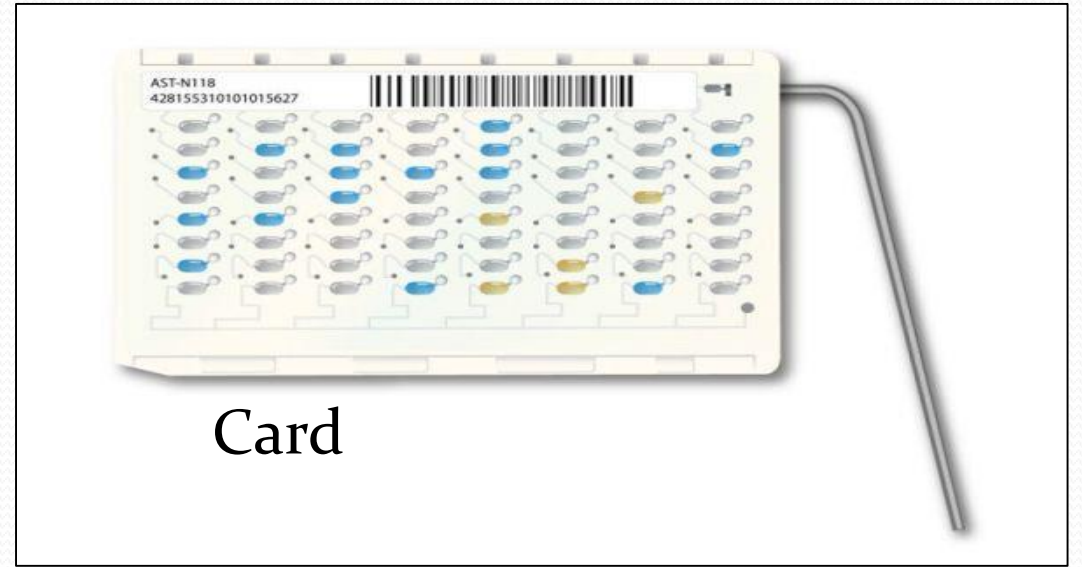


Components :

Dispenser



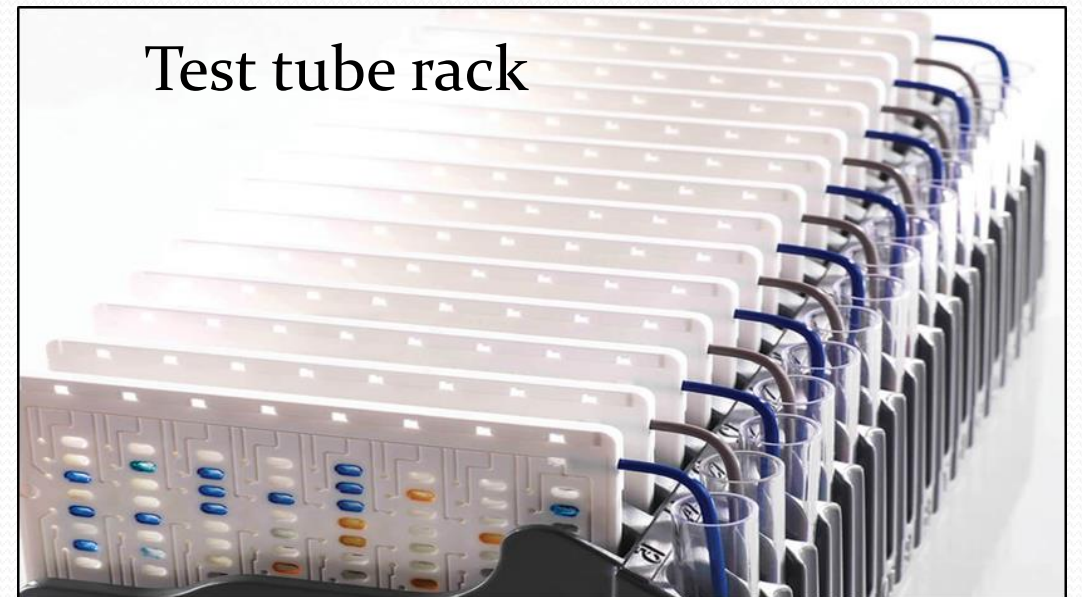
Card



Densichek



Test tube rack



Components :

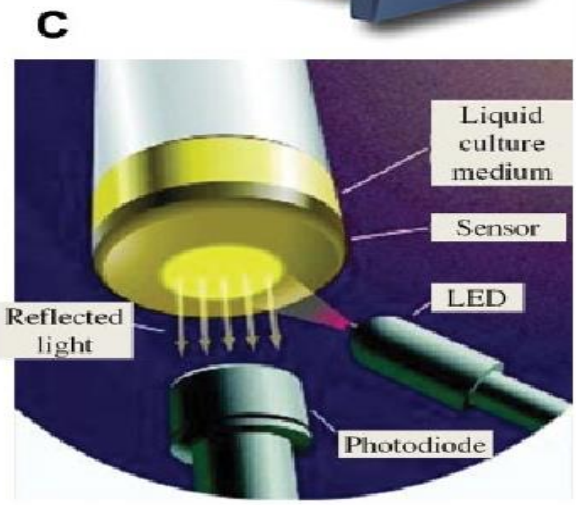
- Dispenser : is used to add saline or other reagents to test tubes containing the microbial samples.
- Densichek: is an automated device used to measure the optical density of microbial suspensions.
- Two cards:
 - 1-Identification card(ID):
 - ❖ 47 biochemical test
 - ❖ Card for GN
 - ❖ Card for GP
 - ❖ Card for Yeast
 - 2-Antimicrobial susceptibility test card(AST):
 - ❖ 22 antibiotics
 - ❖ Minimum Inhibitory Concentration(MIC)

Steps of work:

- 1) Organism isolation
- 2) Gram stain
- 3) Bacterial suspension (2 tubes for ID and AST)
- 4) Measure turbidity (0.5-0.63)
- 5) Insert cards in bacterial suspension tubes
- 6) In to the filling room (transfer the bacterial suspension into the wells of card)
- 7) Transfer the cards into the loading room (diagnostic) 5-10 hrs

BACT system:

- This is the first automated non- radiometric and non invasive culture system that continuously monitors system for culture of bacteria(both aerobic and anaerobic), fungi and mycobacteria
- All these bacteria can be cultured using different media as prescribed
- This is a closed system and works on the colorimetric principle of detection of CO₂ produced by the organisms



Type of bottles:

- Adult (aerobic and anaerobic)
- Pediatric
- Mycosis or Fungi

Steps of work:

- 1) Incubation Chamber: This is where the culture bottles are placed. The chamber maintains optimal conditions for microbial growth by controlling temperature.
- 2) Colorimetric Sensor: Each culture bottle contains a sensor at the bottom that changes color in response to CO₂ production by growing microorganisms.
- 3) Touchscreen Interface: The user-friendly interface allows for easy operation and monitoring of the system.
- 4) Barcode Reader: This helps in tracking and managing samples efficiently by scanning the barcodes on culture bottles.
- 5) Data Management System: The system integrates with laboratory information systems (LIS) for seamless data management and reporting.