

Diagnostic Microbiology

Lab: 1

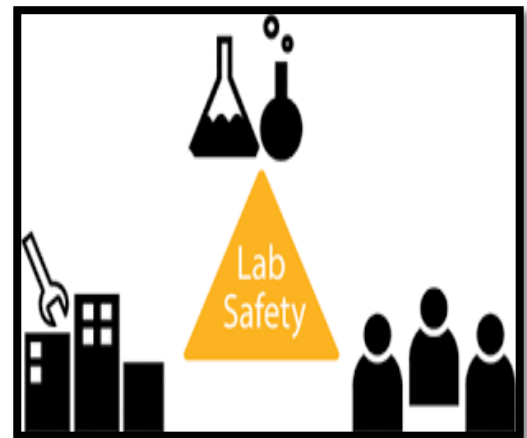
The fourth stage.

Laboratories Techniques.

Biosafety Levels

Biosafety levels are used to identify the protective measures needed in a laboratory setting to protect workers and the surrounding environment from exposure to danger while working with living organisms.

It aims to: provide the highest level of protection and the lowest range of exposure.



Rules and biosafety levels

Risk groups: are guidelines to classify biohazards according to their relative pathogenicity to healthy adults human, and determine its source and how to deal with it, **Includes 4 groups:**

	Risk groups	The level of biological safety for each group
1	(Minimal risk) Risk group 1 Non-pathogenic factors in healthy people and adults (with little or no risk). <i>E.coli</i> , <i>Bacillus subtilis</i> .	Biosafety level 1

2	<p>(Moderate risk) Risk group 2</p> <p>Factors related to human infection and treatment is possible in this case (moderate risk and limited risk of spread). <i>E.coli</i> pathogenic strain, <i>Brucella</i> spp., <i>Salmonella</i> spp.</p>	Biosafety level 2
3	<p>(High risk) Risk group 3</p> <p>Factors that cause serious and fatal infections to humans and their treatment is not easy, especially for immunosuppressed persons (with a high risk for individuals). <i>E.coli</i> O:157 H:7, <i>Mycobacterium tuberculosis</i>, <i>Salmonella typhi</i>, <i>Niesseria meningitides</i></p>	Biosafety level 3
4	<p>(Extreme risk) Risk group 4</p> <p>Factors that are fatal to humans are easily transmitted from person to person. Treatment is difficult and unavailable. Marburg virus, Ebola virus, HIV</p>	Biosafety level 4



Biohazard symbol:

It is used to warn people of the potential for the presence of dangerous biological materials such as :

- Cultures of Pathogens.
- Human Blood and Tissue.
- Corridors leading to the laboratories.

**Laboratory safety considerations:**

1. Wear protective clothing (clean lab. Coat, Gloves, mask, safety glasses).



2. Avoid touching objects (e.g., pencils, cell phones, door handles) while wearing gloves. and -Pencils, labels, or any other materials should never be placed in your mouth.



3. Do not eat food or drink water in the lab. do not use lab glassware as food or water containers.



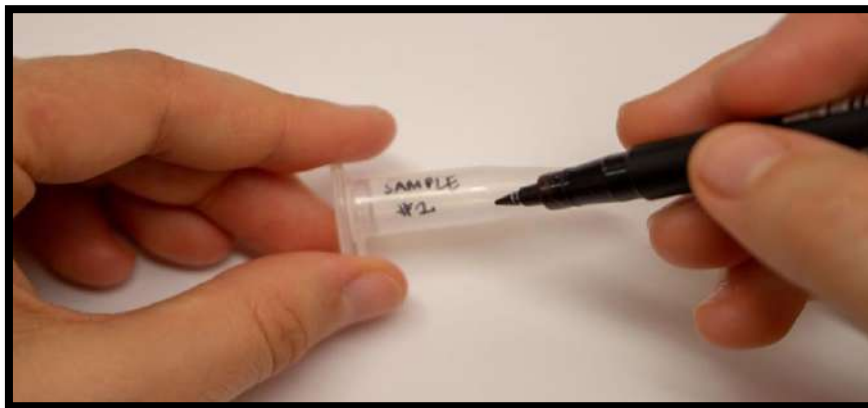
4. Long hair must be tied back or covered to minimize fire hazard or contamination of experiments.



5. Do not take any cultures out of the lab for any reason, All cultures should be handled as potentially pathogenic.



6. Write clear information on each sample.



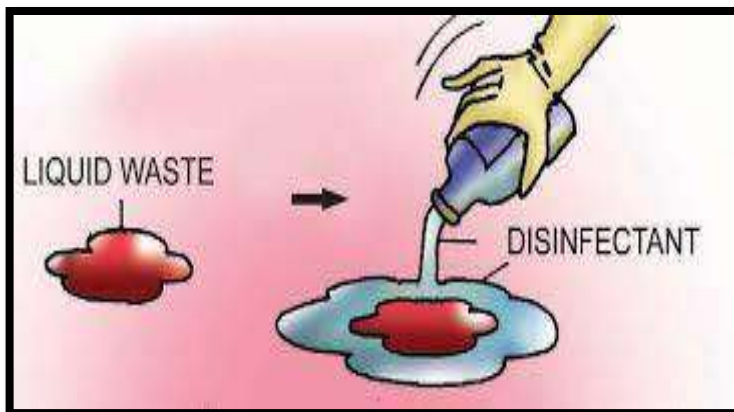
7. wash hands after working with infectious materials.



8. disinfect all instruments immediately after use.



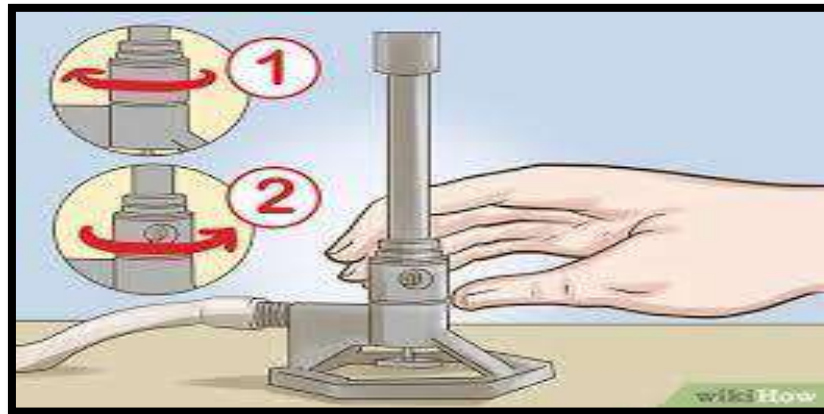
9. disinfect all contaminated waste before discarding.



10. report to appropriate personnel all accidents or exposures to infectious agent.



11. Caution must be taken when using gas burners. Be sure gas burners are turned off when finished.



12. At the end of the lab period return all equipment to its place of origin.

13. Wash your hands very well with soap and water before leave the lab.



STERILIZATION AND DISINFECTION

Sterilization: is the complete killing or removal of all living organism such as cell spore , viruses , fungi , *et al* ...

Disinfection: the destruction or removal of pathogens but not bacterial spores , usually used only on inanimate object .

METHODS OF STERILIZATION

The physical methods of sterilization include:

- Incineration
- Moist heat
- Dry heat
- Filtration

- Ionizing

Physical Methods of Disinfection

- Boiling at 100°C for 15 minutes,
- Pasteurizing at 63°C for 30 minutes or 72°C for 15 seconds
- Using nonionizing radiation

Chemical Methods of Disinfection

- Alcohols
- Aldehyde
- Halogens
- Heavy metals
- Phenolics

Good Luck

Prepared by Asst. Lec. Nadia KH.Mustafa

كلية النور الجامعة
قسم تقنيات المختبرات
الطبية
Diagnostic Microbiology
المرحلة الرابعة
Lab 2/2023



Diagnostic Microbiology

Lab: 2

Assist lecture:Nadia Khalid Mustafa

* **Strategies for choosing appropriate diagnostic methods**

- In diagnosing infections, the rapid identification at an early stage of the disease is critical for a favorable outcome.
 - it is important that exact information be obtained on the stage of the disease rapidly in order to choose and initiate the appropriate therapy.
- In recent years many new techniques have been added in the diagnostic tools. molecular biology as well as in the application of nucleic acid technology to the study of the epidemiology of human infection, where the diagnosis of viruses using PCR and ELISA.
- Lastly, many rapid culture methods are coming up to achieve faster bacterial diagnosis like Vitek2 System.

*Interaction between antigen - antibody

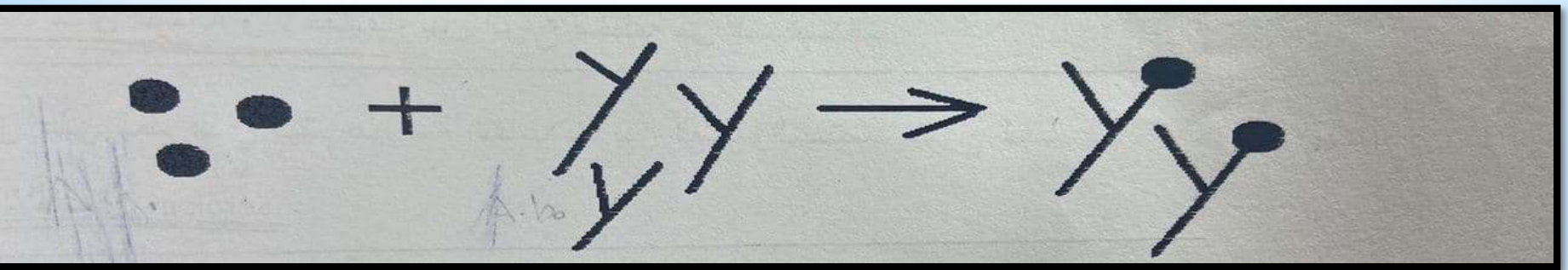
The science concerned with the interaction of the antibody with the antigen outside the body (Invitro) of an organism is called **serology**.

Types of serological reactions:

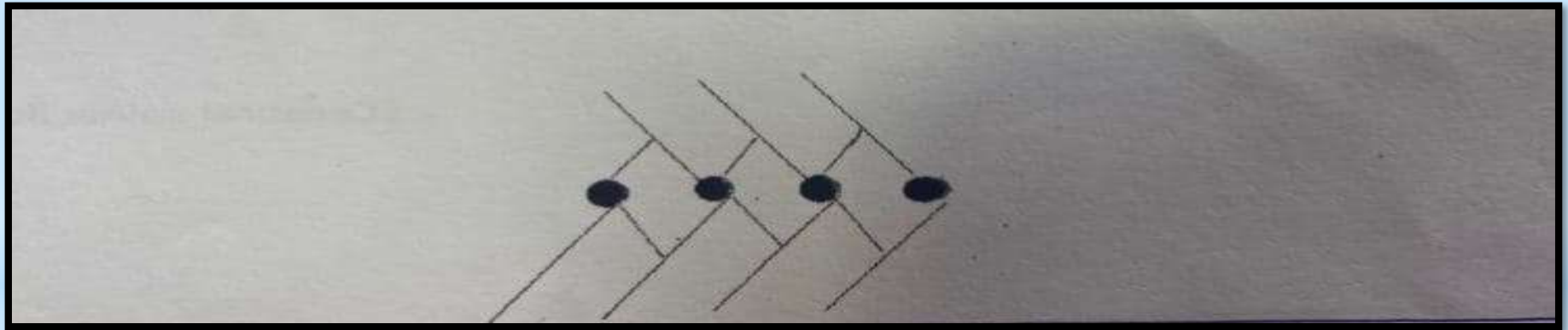
1. Agglutination.
2. Precipitation.
3. Enzyme-Linked Immune Sorbent Assay (ELISA).
4. Immuno electrophoresis.
5. Immuno fluorescens.
6. Radio Immuno Assay (RIA).

* The stages of interaction between antigen and antibody

The first stage: It is done within seconds by mixing the antigen with the antibody to form the first immune complex.

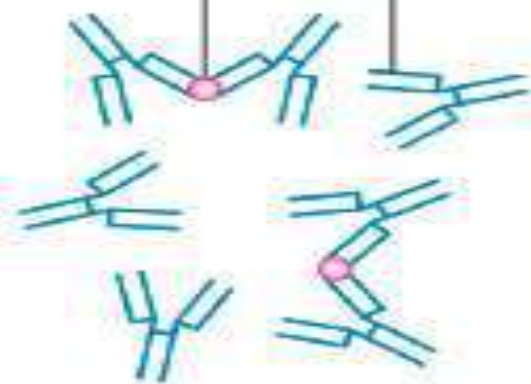


The second stage: It is done within minutes to hours, in which a Lattice appears resulting from grouping the complexes formed in the first stages and produces large sedimentations of complexes.

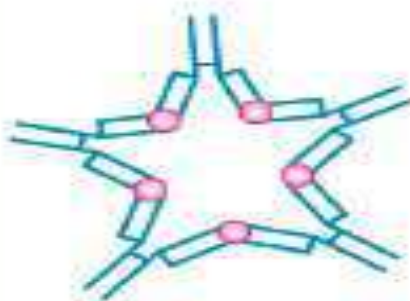


Prozon: it is the phenomenon of the presence of very high concentrations of antibodies in the serum, which leads to the absence of the clumping lattice clearly, and thus it is a false negative result, In the sense that the first stage interaction only occurs and the second stage does not occur.

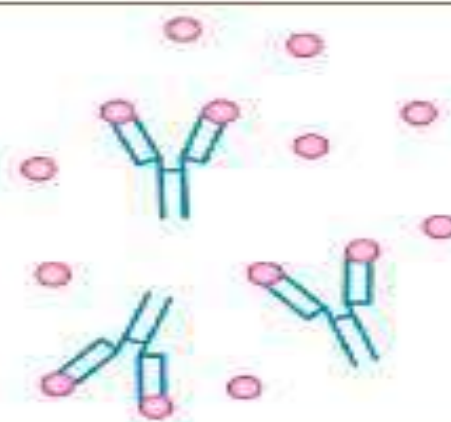
Antigen Antibody



Antibody excess
(prozone)



Zone of equivalence
(Lattice formation)



Antigen excess
(post zone)

* Agglutination test

It is the binding process between an antibody and an insoluble antigen to form an immune complex that can be seen directly.

Types of Agglutination test:

1. Direct Agglutination test:

2. Indirect Agglutination test:

3. Haemagglutination Inhibition test:

*Practical part for Agglutination test:

- Put 50 μ l serum and one drop of each positive and negative control into separate circles on the slide test.
- Shake the reagent gently before using and add a drop of this reagent to the serum and controls.
- Mix both drops with a stick.
- Rotate the slide for (2 – 5 min) and read the result by the eye and the microscope.

*ELISA

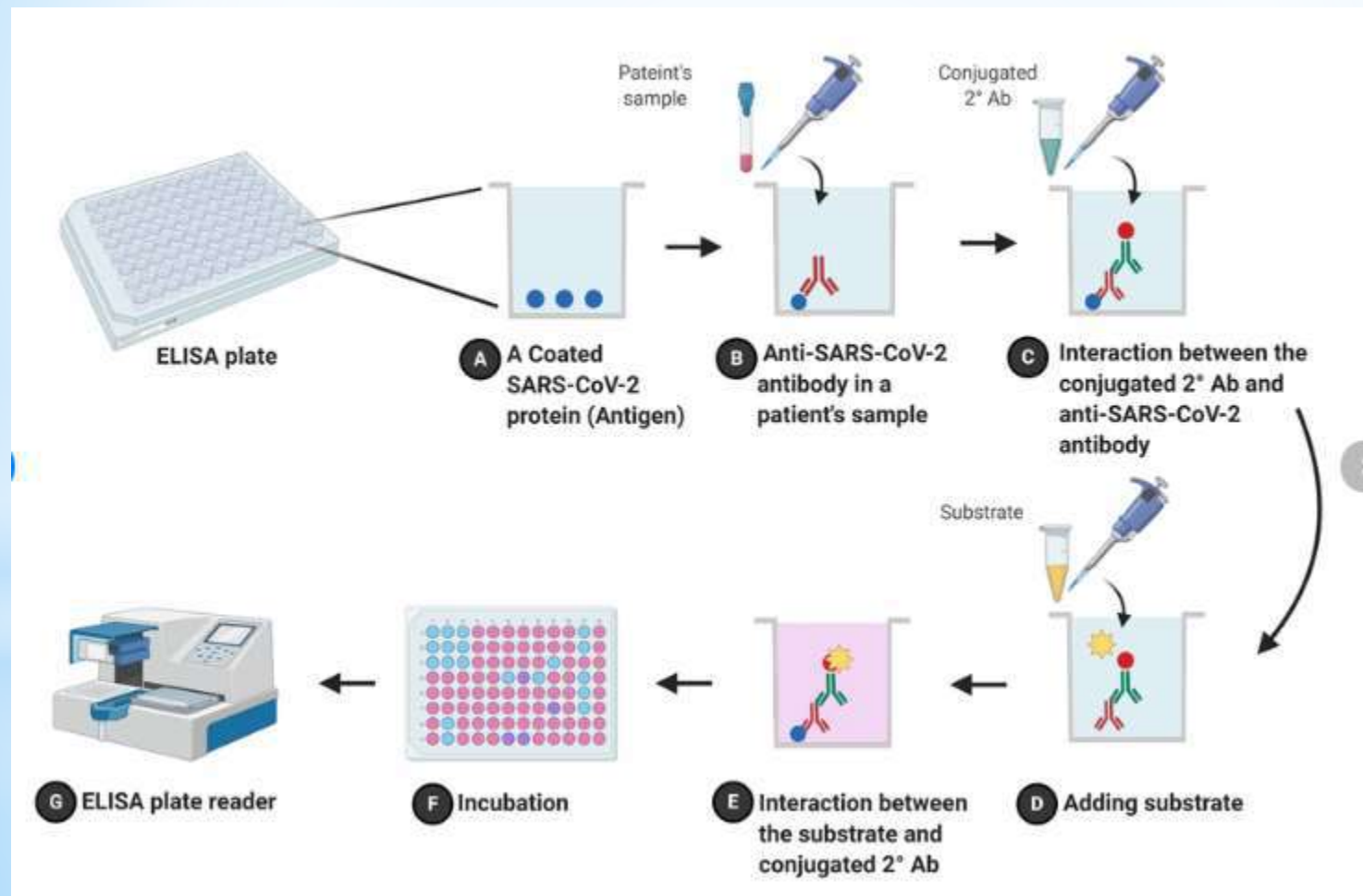
A highly sensitive immune biochemical technique in which the Ag – Ab complex is detected, where one of them is tagged with an enzyme, and when the non-color reagent is added, the enzyme gives a color reaction.

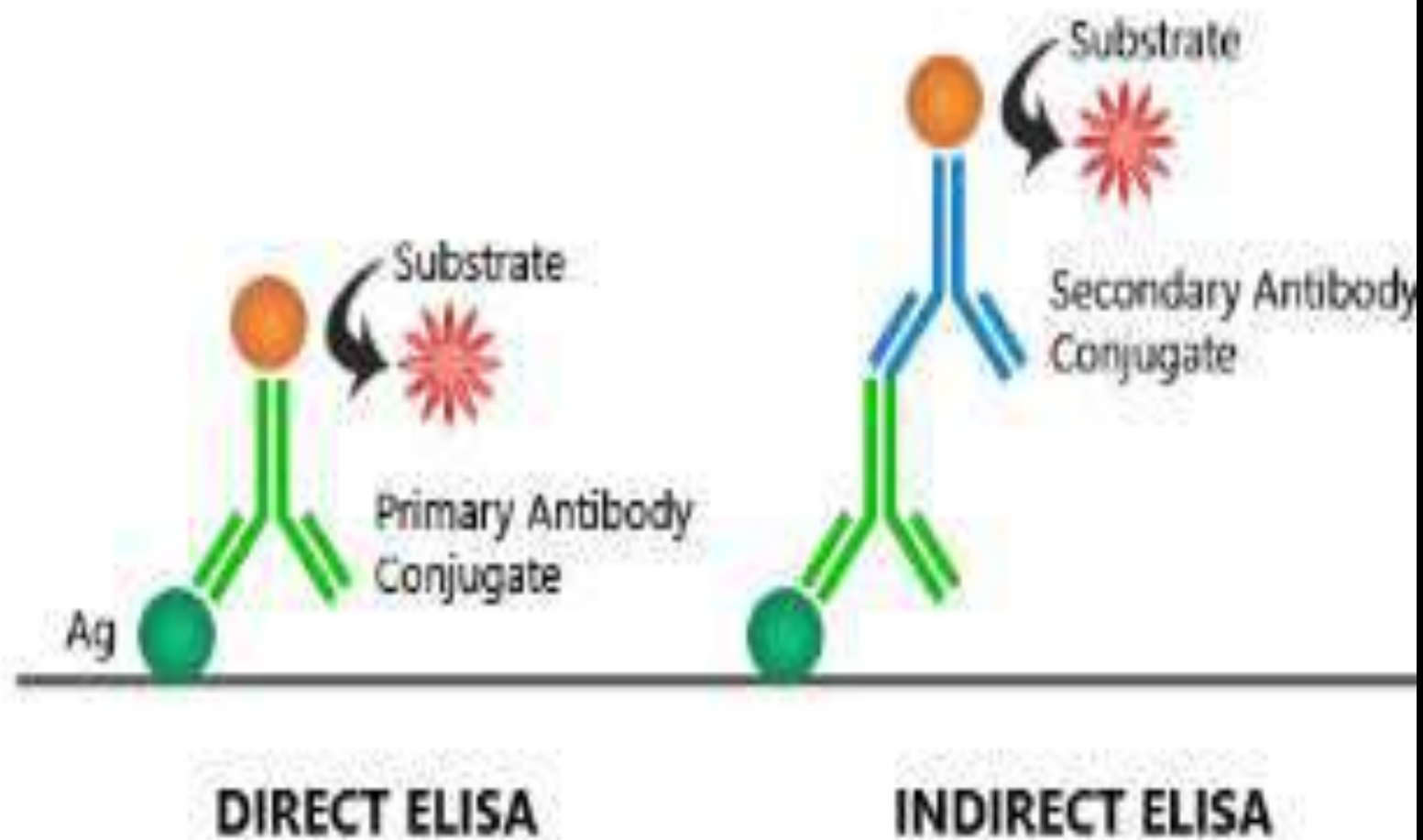
The importance of the ELISA test:

- 1.Diagnosis of viral, bacterial and parasitic diseases.
- 2.Detection of antigens.
- 3.Detection of antibodies.

*Types of ELISA:

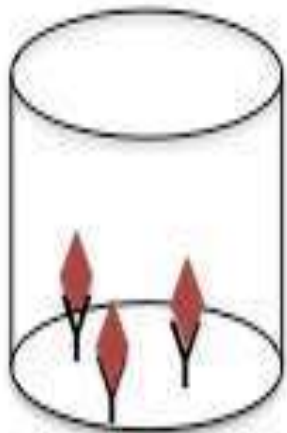
Indirect ELISA(detection of Antibody)



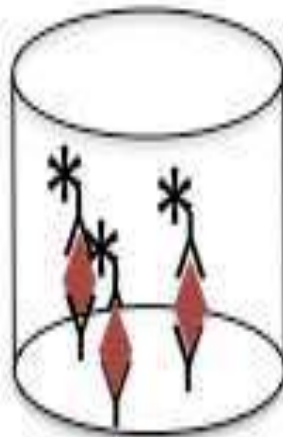


*Direct ELISA (Sandwich, detection of Ag) :

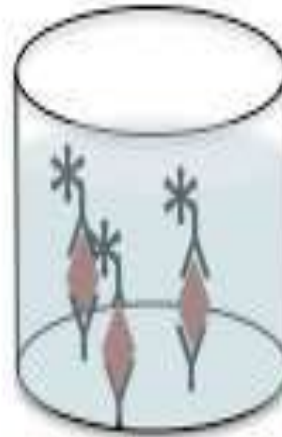
1. Add sample to capture Ab-coated plate.



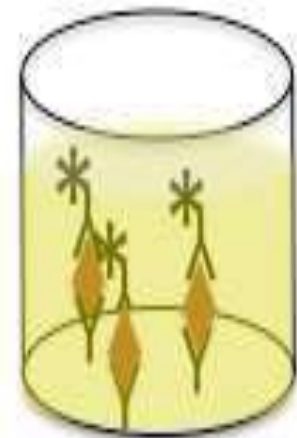
2. Add enzyme-linked detection antibody.



3. Add colorimetric substrate and develop.



4. Read absorbance values and calculate analyte concentration.

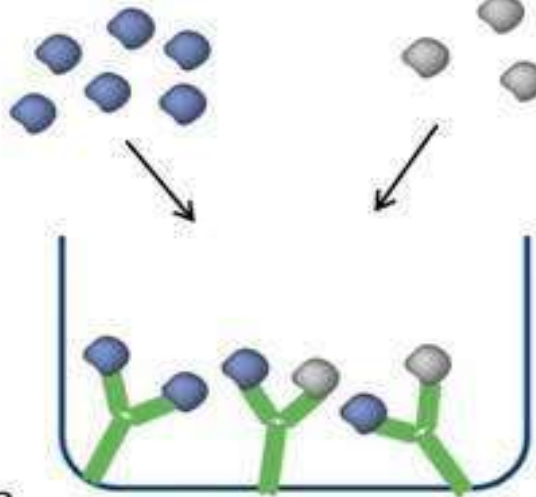


*Radioimmunoassay (RIA)

- the antibody or antigen is attached to a radioactive isotope such as iodine, It is not commonly used because of its danger.
- It is used to measure hormones and cancer markers.
- Their types are: competitive RIA and Sandwich non-competitive RIA.

Infected serum sample
(containing unlabeled
Hepatitis B Surface antigen)

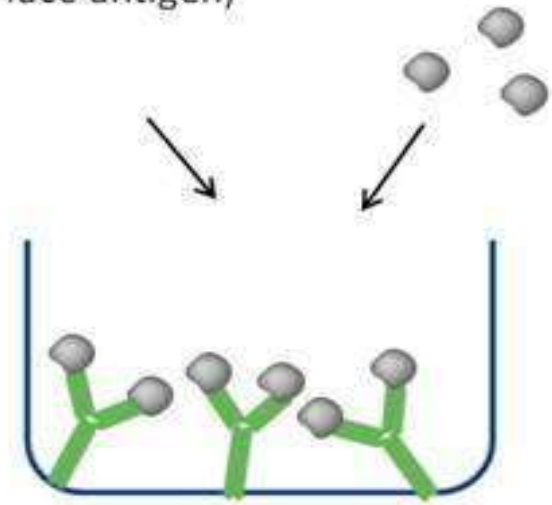
^{125}I Hepatitis B
Surface antigen



a

Uninfected serum
(no Hepatitis B
Surface antigen)

^{125}I Hepatitis B
Surface antigen



b

Thanks
for
Listening

كلية النور الجامعة
قسم تقنيات المختبرات
الطبية
Diagnostic Microbiology
المرحلة الرابعة
Lab 3 /2023



(PCR, HPLC, Vitek 2 system)

Diagnostic Microbiology

Lab: 3

Assist lecture: Nadia Khalid Mustafa

PCR

The Polymerase Chain Reaction (PCR): is a biochemical technology in molecular biology used to amplify a single of DNA enzymatically outside the body of an organism, generating thousands to millions of DNA.

Developed in 1983 by kary mullis.

The principle of PCR technology: It depends on the mechanism of DNA replication inside the body of the organism, which is:

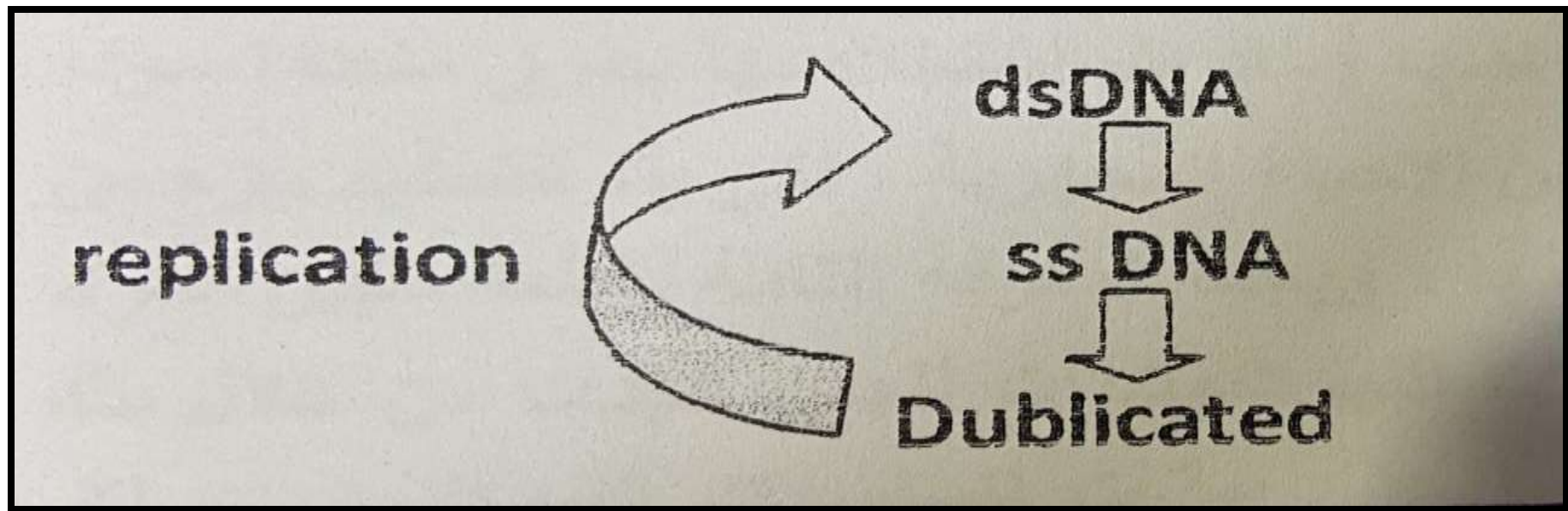


Figure (1): The principle of DNA replication in the body (in vivo)

PCR technology requirements:

- **DNA template** that contains the DNA region (target) to be amplified.
- Two **primers** that are complementary the DNA target.
- **Tag DNA polymerase** isolated from *Thermus aquaticus* bacteria.
- Deoxynucleoside triphosphate (**dNTPs**).
- **Buffer** solution.

The reaction is carried out using a Thermocycler.



Figure (2): Thermocycler

PCR cycle:

Consist of three steps:

1- Denaturation : the double-stranded DNA is heated to 95 °C to separate into single strands.

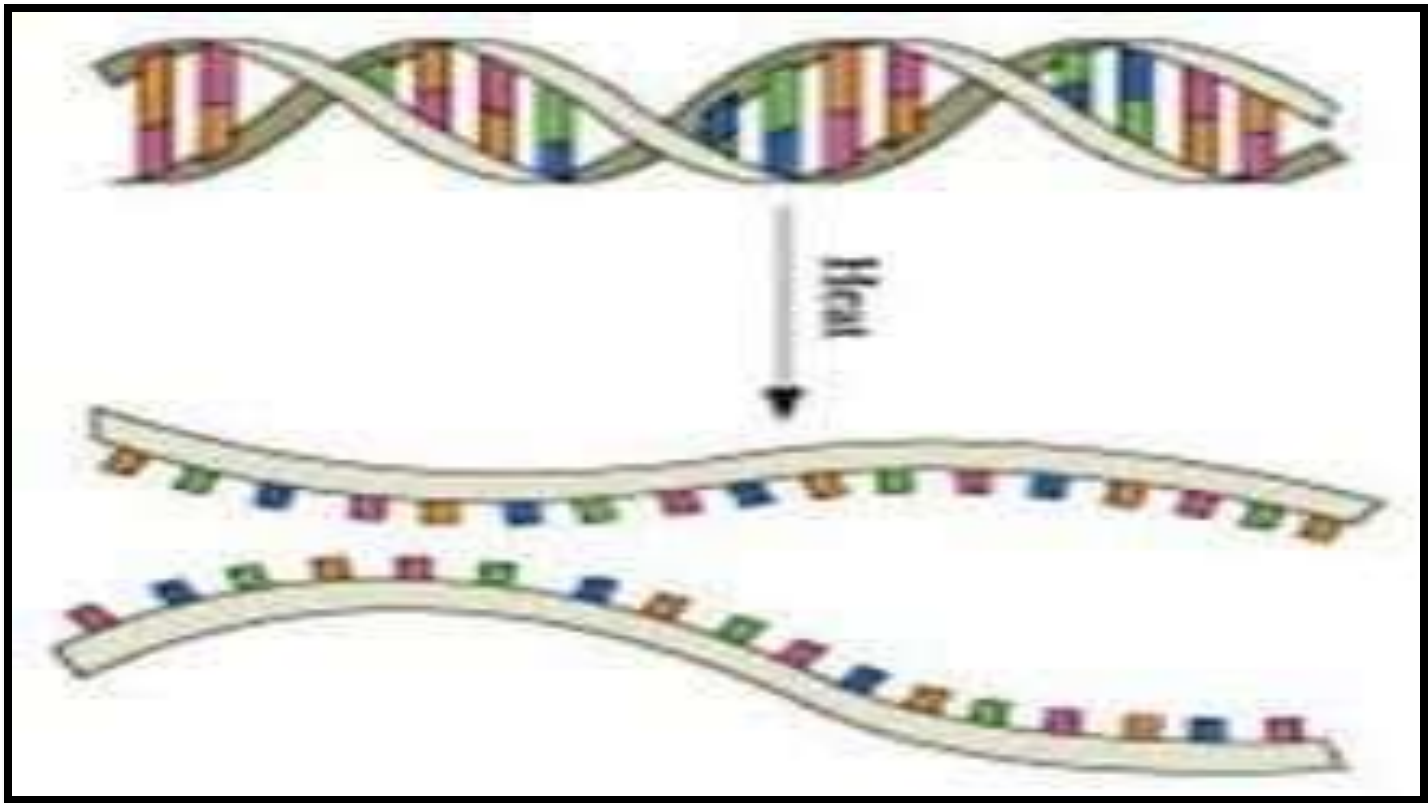


Figure (3): Denaturation of DNA

2- Annealing: Cooled to 50 °C , the primes bind or anneal with separate DNA strands.

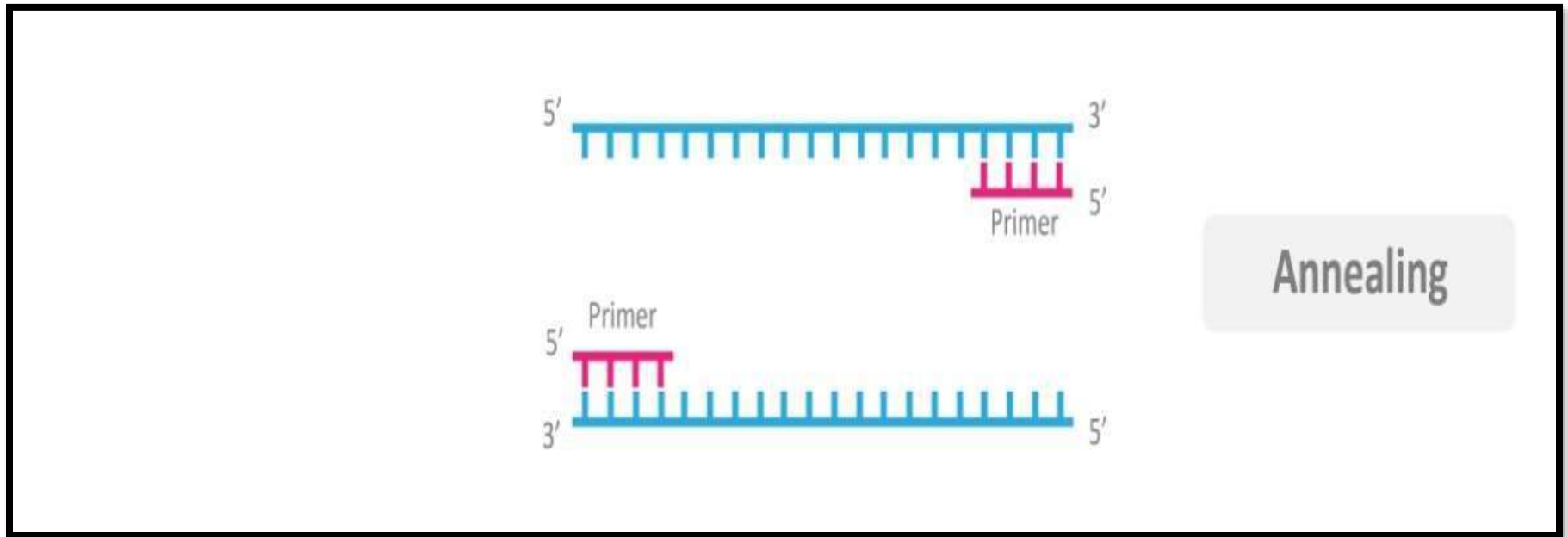


Figure (4): Annealing of primer

3- Elongation: at 70 °C the Tag DNA polymerase bind with primer.

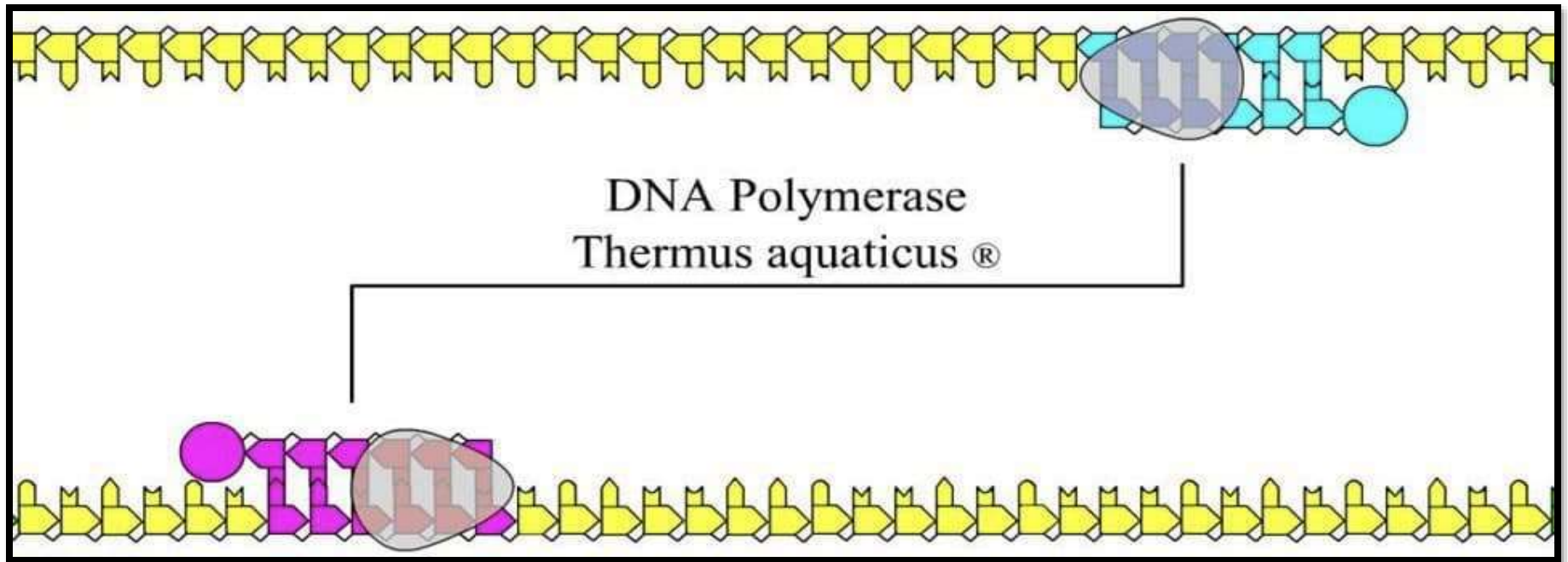


Figure (5): DNA polymerase bind with primer

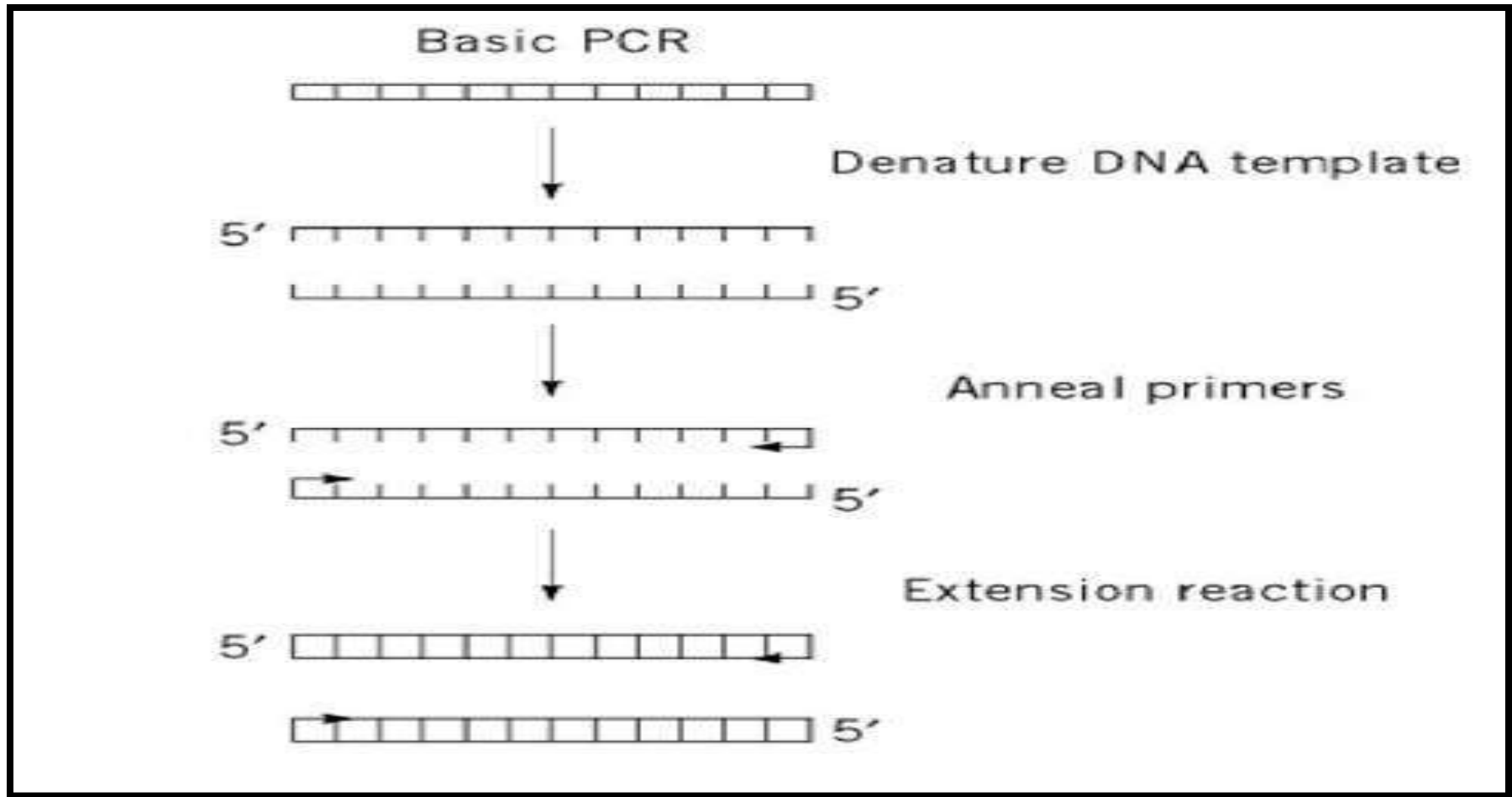


Figure (6): DNA replication cycle using PCR

The DNA amplification products are detected using electrophoresis.

Some applications of PCR technology:

- Detection of the desired gene.
- Detection of genetic mutations.
- Detection of difficult to grow microorganisms in culture media.

HPLC

High-performance liquid chromatography (HPLC):

Technique in analytical chemistry used to separate and identify each component in a mixture.

It Depend on pumps to pass a liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.

The importance of chromatography:

HPLC has been used for:

- **manufacturing** (e.g., during the production process of pharmaceutical and biological products).
- **research** (e.g., separating the components of a complex biological sample, or of similar synthetic chemicals from each other).
- **medical** (e.g., detecting vitamin D levels in blood serum) purposes.

What are the principle of HPLC

The principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase and a stationary phase . Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time “on-column”. Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved..

High Performance Liquid Chromatography (HPLC)

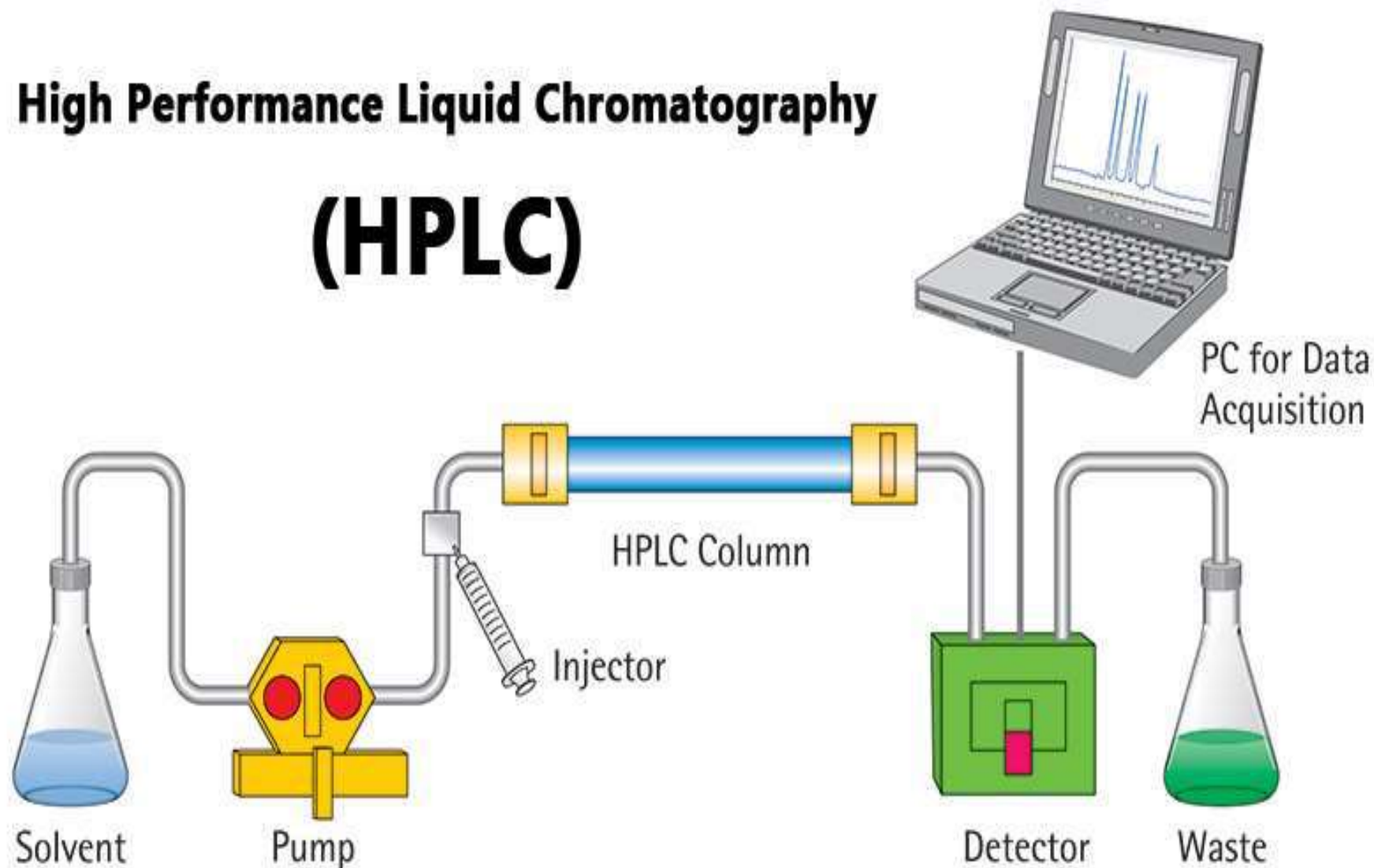


Figure (7): HPLC

Vitek 2 System

The traditional methods used to detect and diagnose microorganisms from polluting sources. They are often dependent on:

- the time consumed for growth in the culture medium,
- followed by isolation and biochemical and sometimes serological diagnosis.

While this device provides automatic tests, it is able to quickly diagnose within a few hours instead of the days required for classical methods.

Vitek 2 System: is a fully automatic and commercial diagnostic device used in microbiology to identify the types of germs within a short period, and this device has become the focus of attention of clinical microbiology laboratories, as it allows accurate and rapid diagnosis of bacteria and yeast relevant medical.

The device is developed by the French company Biomerieux.



Figure (8): Vitek 2 system device

The importance of Vitek 2 system:

- it identifies the type of bacteria automatically in the device without the need for any external tests.
- It used for antibiotic susceptibility test.
- it has the ability to diagnose 500 bacterial species..

It's distinguishes from the PCR device is that it only recognizes viable cells.

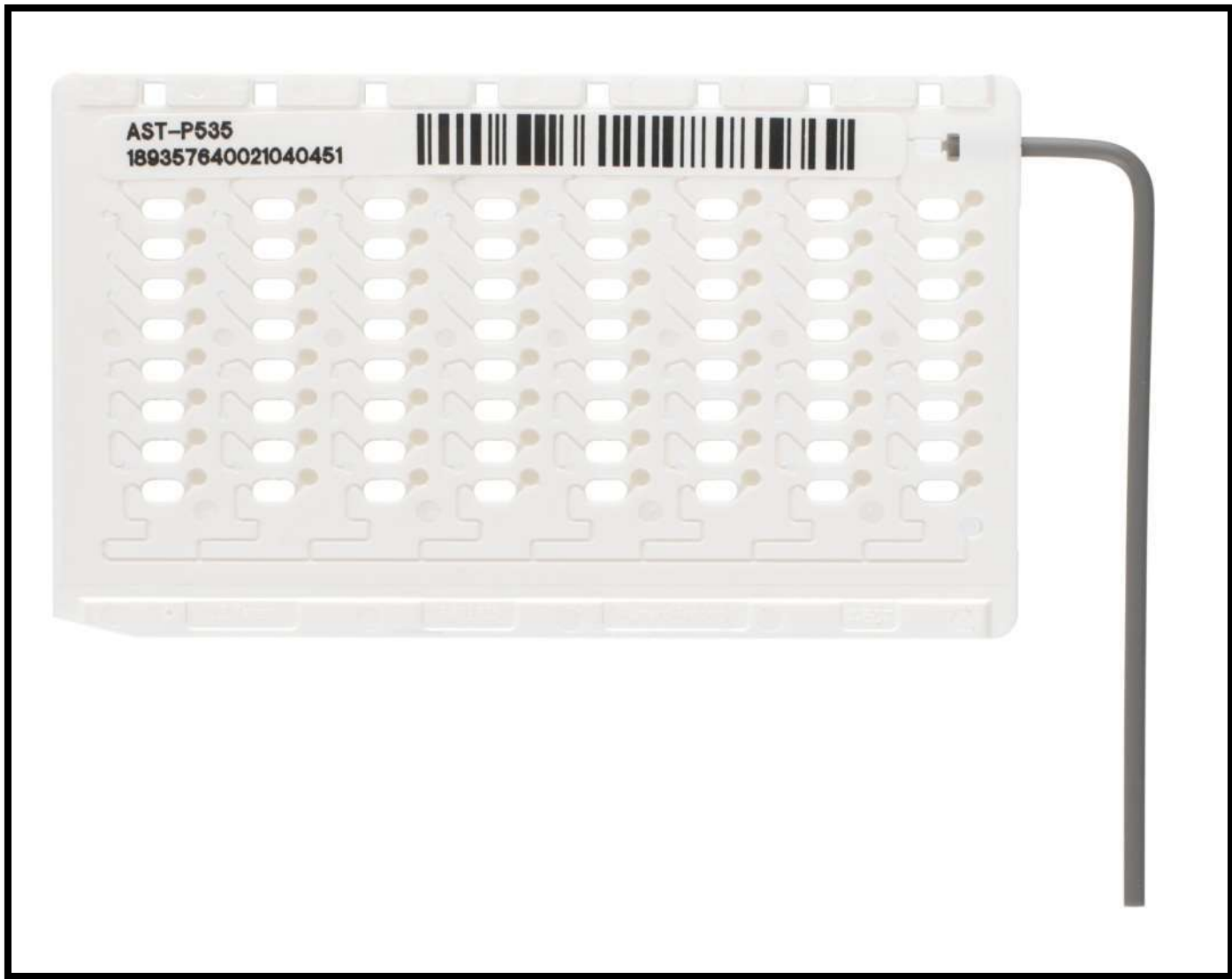


Figure (9): AST card for the Vitek2 system device

Types of cards:

- 1- Gram negative card (GN card):** it contains 47 biochemical tests. diagnostic results are available in approximately 10 hours or less.
- 2 - Gram-positive card (GP card):** There are 43 biochemical tests that show diagnostic results in about 8 hours or less
- 3- Hemophilus / Nesteria Card (NH card).**
- 4- Corynebacteria and anaerobic diagnostic card (ANC card).**
- 5- Bacillus Diagnostic Card (BCL card).**
- 6- Corynebacteria Diagnostic Card (CBC card).**
- 7- Yeast card (YST card).**

The Vitek 2 system device is used as follows:

1- Preparing the suspension: use a sterile wooden rod or swab to transfer the colonies from a pure, modern culture and suspend them in 3ml of normal saline inside a plastic test tube.

2- Inoculation of the card: Transfer the suspension and the card to the device holder, which is a special rack that contains slots for placing the diagnostic card.

3- Optical System.

4- results.



Figure (10): cards for the Vitek2 system device

Thanks
for
Listening

كلية النور الجامعة
قسم تقنيات المختبرات الطبية
Diagnostic Microbiology
المرحلة الرابعة
Lab 4 /2023



(Aerobic requirements of microorganisms

Diagnostic Microbiology
Lab: 4

Assist lecture: Nadia Khalid Mustafa

Aerobic requirements of microorganisms

Oxygen is an important environmental factor for the growth and spread of the microorganism, the microorganism that is able to grow in the presence of atmospheric oxygen is called **Aerobic M.O.** , a microorganism that is able to grow in the absence of oxygen is called **Anaerobe M.O.**

The difference in the oxygen needs of the microorganisms reflects the difference in the **biooxidative enzyme system** present in the different species.

Therefore, the microorganisms can be divided into five main groups according to their need for oxygen:

1- Obligate aerobes:

Dependent on Oxygen completely to grow, that means the Oxygen is the final receptor for the electron in the electron transport chain during aerobic respiration, e.g. : *Staphylococcus aureus* , *Pseudomonas* spp.

2- Facultative anaerobes:

Do not need oxygen to grow, but it grows better with it, in the case of **the presence of oxygen**, it follows the path of aerobic respiration and the oxygen is the final receiver of the electrons,

but in **the absence of oxygen**, it takes:

either the **fermentation path** and uses organic compounds as the final electron acceptor

or takes the **anaerobic respiration path**, using inorganic compounds such as nitrates NO_3^{-1} or sulfates SO_4^{-2} as the final acceptors of the electron, e.g. : *E.coli*.

3- Aerotolerant anaerobes:

It grows well with or without oxygen , it does not depend on it to produce energy, but it depends only on fermentation and it is called **obligate fermenter**. e.g. : *Lactobacillus bulgaricus* , *Streptococcus pyogenes*, *Enterococcus faecalis*.

4- Obligate anaerobes:

It does not tolerate oxygen and dies with its presence, albeit in a very small percentage, as it is toxic to it, So it need oxygenless conditions to grow because its oxidizing enzymatic system needs the presence of molecules other than oxygen as the final receptor of the electron, so it gets energy from the **fermentation** processes or the **anaerobic breath**, e.g: *Clostridium pasteurianum* , *Bacteriodes fusobacterium*.

5- Microaerophiles:

It needs very low levels of oxygen, not exceeding 2 to 10%, as it is destroyed at normal levels of atmospheric oxygen 20%.
e.g.: *Helicobacter pylori* , Campylobacter.

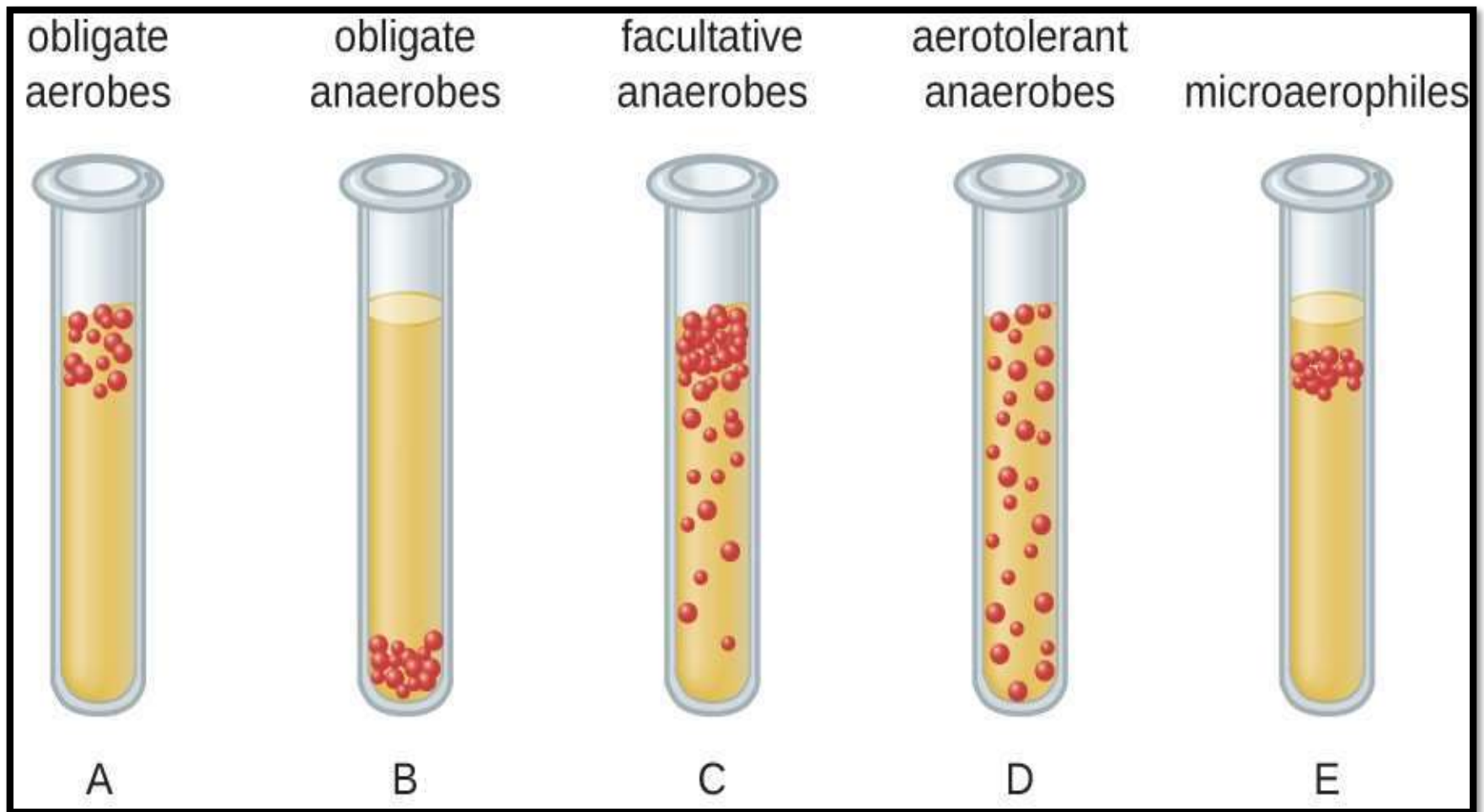
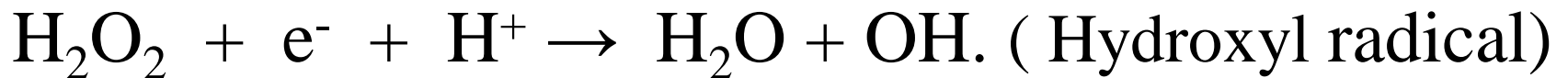
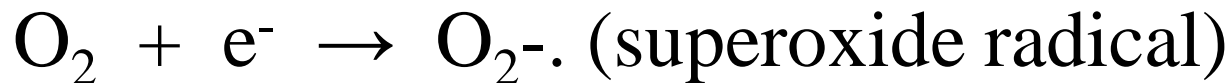


Figure (1): The nature of the growth of the microorganism according to its need for oxygen

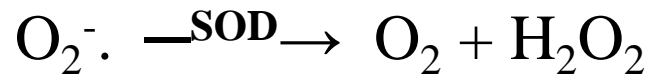
The effect of oxygen on microorganisms is through the formation of toxic oxygen derivatives that inhibit proteins,

Toxic oxygen derivatives are formed by rapidly gaining electrons and reducing them as shown by equations:



How do microorganisms protect themselves from toxic oxygen reducing products:

Facultative anaerobes and obligate aerobes: possess an enzyme Superoxide dismutase (SOD) that destroys the roots superoxide radicals it also possesses an enzyme Catalase or peroxidase which break down the hydrogen peroxide H_2O_2 .



Aerotolerant : Some of them possess an enzyme SOD and not have Catalase.

Obligate anaerobe: It does not have an enzyme SOD and Catalase, so it cannot tolerate oxygen and you die with it.

Methods for providing anaerobic conditions:

1- Use of **special media** containing reducing agents such as: Thioglycolate, cysteine.

2- The use of **anaerobic jar** by withdrawing oxygen from it by using vaccum pump and replacing it with nitrogen gas and CO₂

3- Using a **Gaspak jar** that contains hydrogen that reacts with oxygen to form water droplets, and it is placed in a glass or metal jar.

Anaerobic jars eliminate the oxygen for anaerobes to grow.

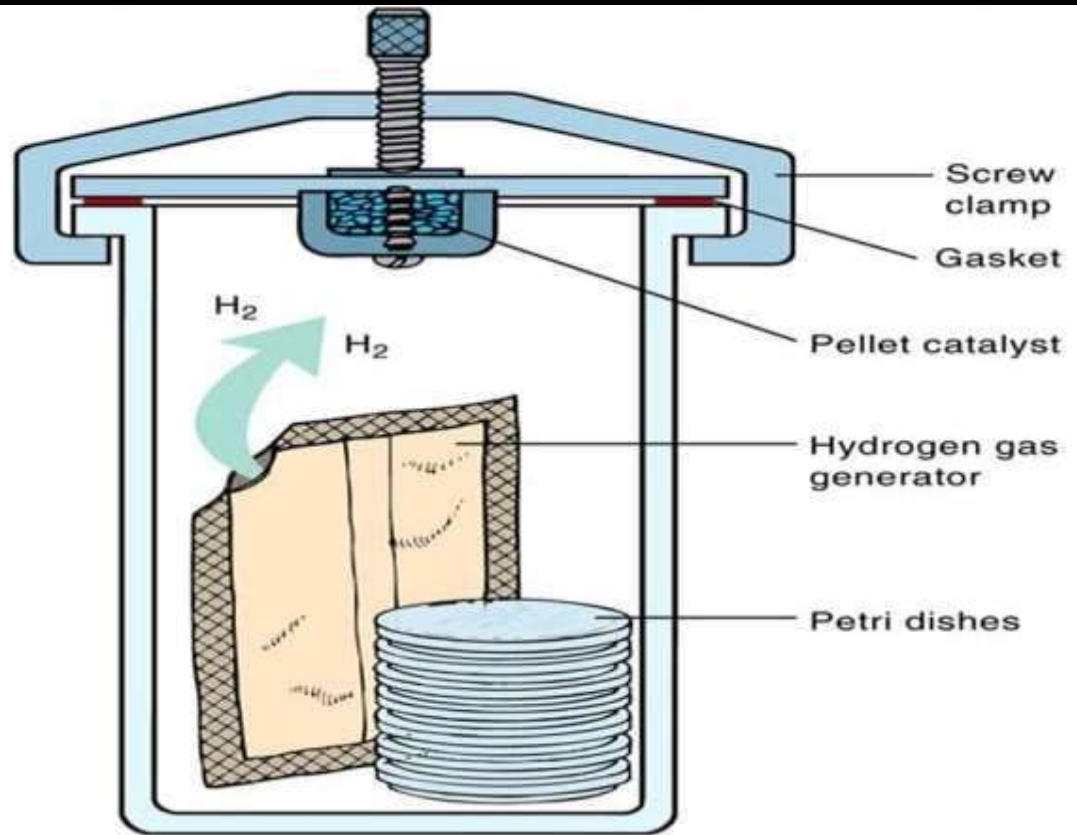


Figure (2): Gaspak jar

4- Use the **Candle- jar**: used a burning candle is placed inside the glass or metal container that consumes oxygen after closing the container and provides 5 – 10% of CO₂

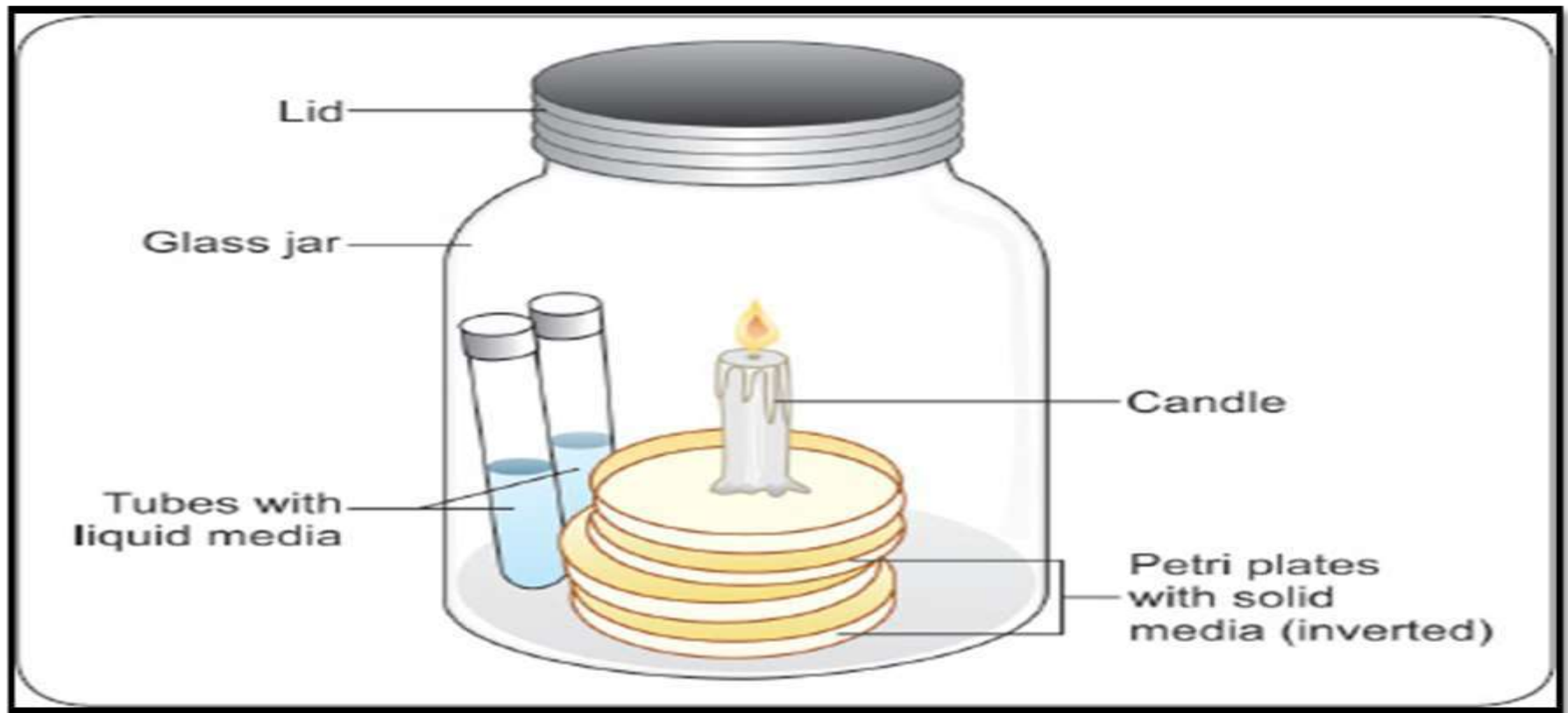


Figure (3): Candle- ja

5- Use of **anaerobic incubators**.



Figure (4): anaerobic incubators

practical part:

A- The nature of the bacterial response to oxygen can be determined using a method Shake-tube inoculation:

- Inoculation of the microorganism in a tube containing a dissolved solid culture medium.
- Mix well to ensure the spread of the inoculum into the culture medium.
- Leave the medium to harden.
- incubate the medium.
- Record the growth distribution pattern in the medium.

B- To provide anaerobic conditions in broth medium, follow the following:

- Inoculation of the culture broth medium with the microorganism.
- added a layer of **paraffin oil** to provide anaerobic conditions.
- Incubate tubes at 37 for 48 hours.
- Check growth: (+ turbidity of the medium),
(- no turbidity of the medium).

C- To provide anaerobic conditions in agar medium, follow the following:

- Inoculate the plate agar with bacteria.
- Place the plate agar inside the jar.
- Place the candle inside the jar and close it.
- Place the jar inside the incubator.

Thanks
for
Listening



(Microscope and Bacterial Staining)

Diagnostic Microbiology

Lab: 5

Assist lecture:Nadia Khalid Mustafa

The basic procedures in the laboratory diagnosis of infectious diseases is as follows:

- 1- **Direct examination** of patient specimens for the presence of etiologic agents.
- 2- **Growth and cultivation** of the agents from these same specimens.
- 3- **Analysis** of the cultivated organisms and performing **sensitivity tests**.

What is a Microscopy:

Microscopy is defined as the use of a microscope to magnify objects too small to be visualized with the naked eye.

Because most infectious agents cannot be detected with the naked eye, microscopy plays an important role in the laboratory.

microscope parts:

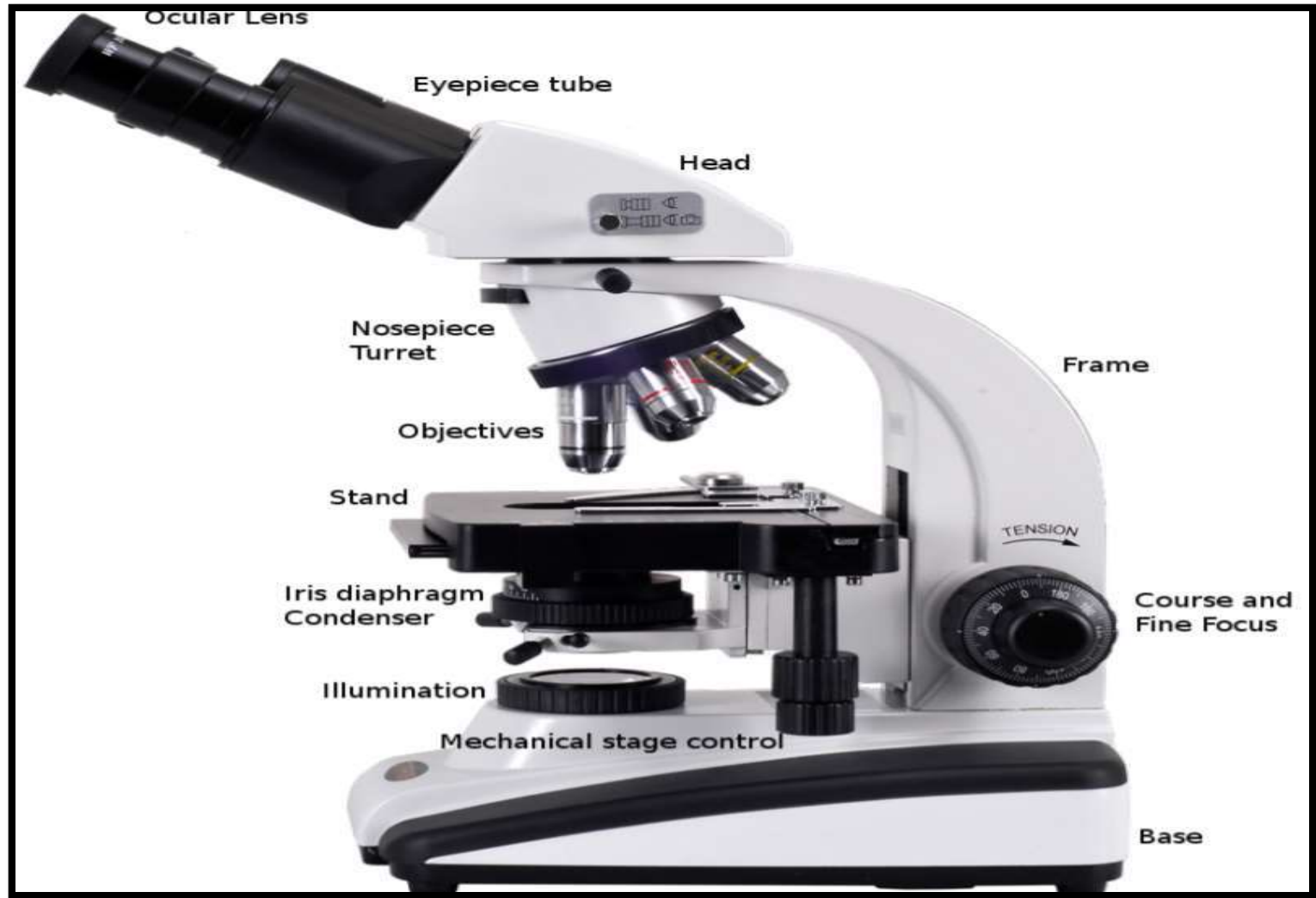
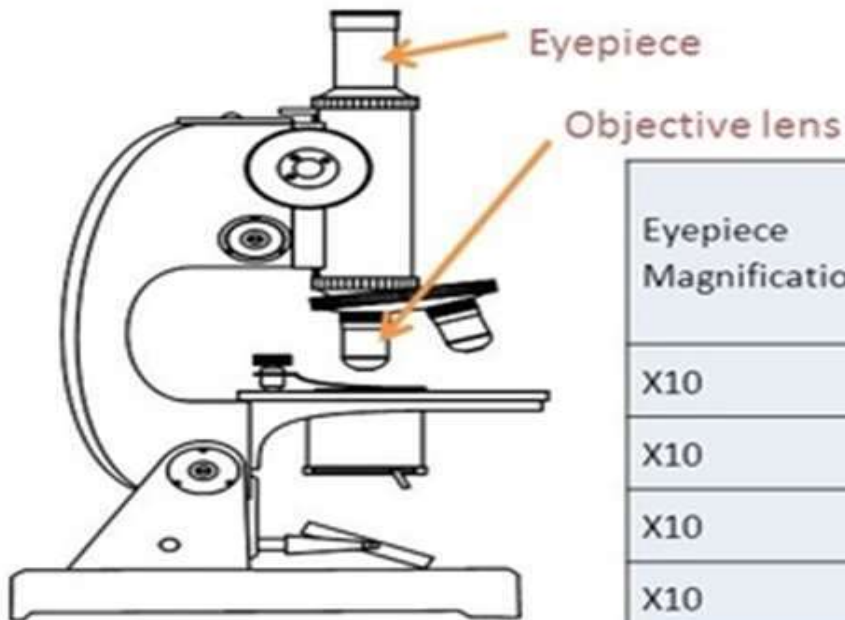


Figure (1) : microscope parts

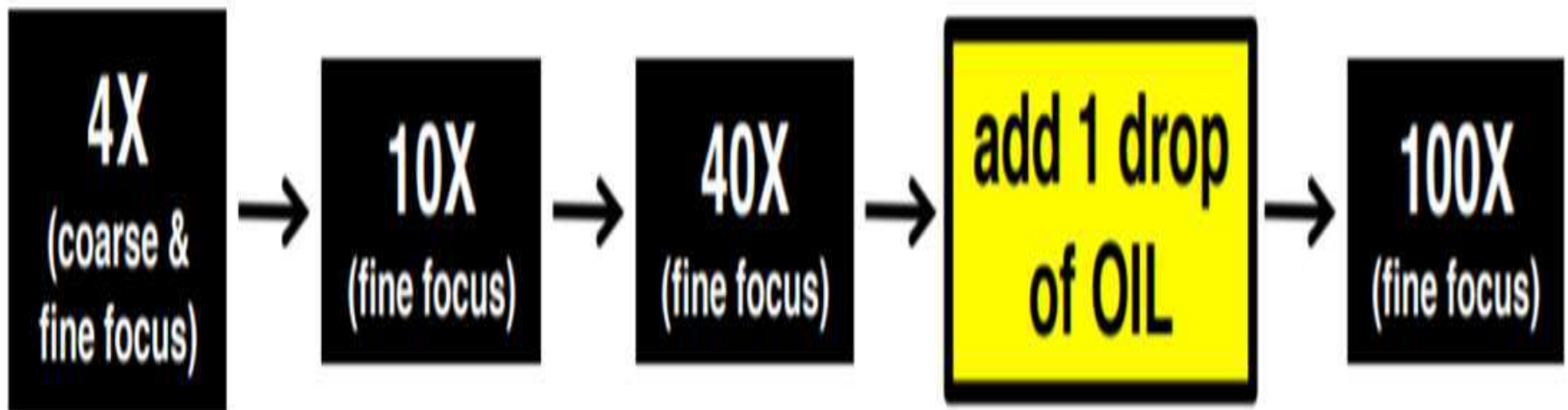
Our microscopes have 10X oculars lenses and 4X, 10X, 40X and 100X objectives lenses ,
the magnification is = (power of the oculars) multiplied
by (power of the objectives)

How do we find the overall magnification of a light microscope?



Eyepiece Magnification	Objective Magnification	Overall Magnification
X10	X4	40
X10	X10	100
X10	X40	400
X10	X100	1000

to achieve the level of resolution desired with 1000x magnifications, oil immersion must be used, oil is used to fill the space between the objective lens and the glass slide onto which the specimens has been affixed.



Applications of Microscopy:

- 1- Rapid identification of organisms.
- 2- Detection of different organisms present in the same specimen.
- 3- Detection of organisms not easily cultivated in the laboratory.

Cleaning a Microscope:

- 1- Lower stage.
- 2- Remove slide, turn the power off.
- 3- Wipe oil from all surfaces and 100X with lens paper.
- 4- With the second piece of lens paper, moistened with alcohol, wipe all surfaces.
- 5- Wipe surfaces with a new dry piece of lens paper.

Bacterial Staining

Bacterial Staining:

Living bacteria are generally colorless and difficult to view with bright-field microscopes.

staining is necessary in order to:

- 1- make them visible for observation of intracellular structures .
- 2- as well as overall morphology.

Smear Preparation:

A bacterial smear is a dried preparation of bacterial cells on a glass slide. The smear use to preparing a specimen for staining.

Bacterial smear Procedure:

- 1- Draw a circle on the bottom of the slide.
- 2- Spread the bacteria from (liquid or agar plate) on the surface slide then mixed into a small drop of water on the slide.
- 3- Let it to air dry.
- 4- Pass the slid three times over the flame to fix the organisms.

Fixation causes the specimen to:

- adhere the specimen to the glass slide.
- kill the microorganisms.
- generally preserve their shape and size.

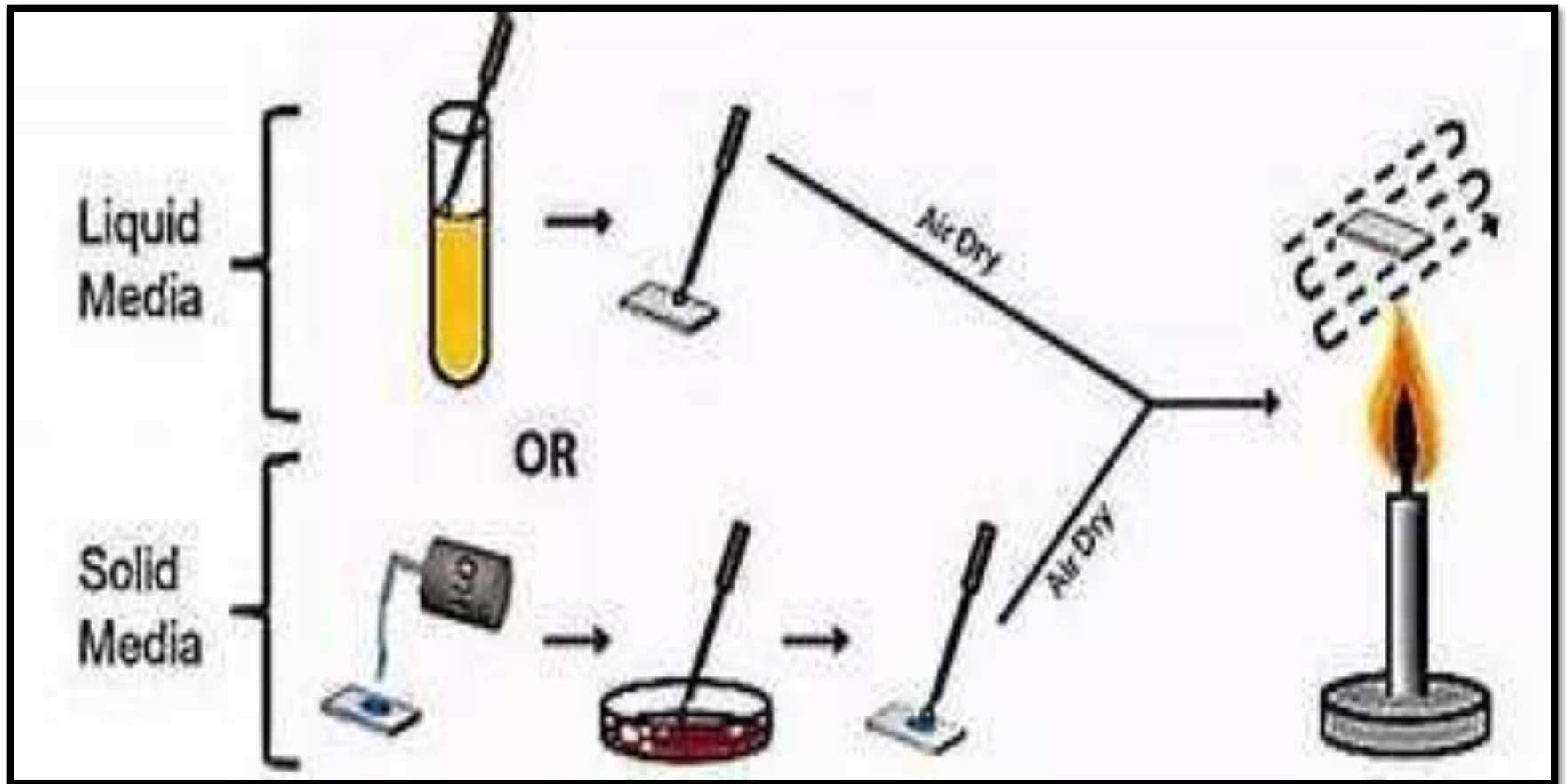


Figure (2): Procedure for making a bacterial smear.

The importance of Gram stain:

1- It divides bacteria into two groups: gram negative and gram positive.

2- Use for classification and differentiation of bacteria.

The gram stain reagents:

- 1- **Primary stain (Crystal violet)**: Its function is to stain all cells.
- 2- **Gram's iodine (Mordant)**: increases the cells' affinity for a stain.(crystal-violet–iodine complex (CV-I)).
- 3- **Decolorizer (Ethyl Alcohol, 95%)**: serves as lipid solvent.
Its action is determined by two factors:
 - the concentration of lipids and the thickness of the bacterial cell walls.
- 4- **Counterstain (Safranin)**: If the primary stain is removed, the cells will be stained with safranin.

Gram Stain Procedure:

- 1- Apply the primary stain (Crystal violet) for 1 min to the smear.
- 2- Wash with tap water
- 3- Apply the mordant (Gram's iodine) for 1 min.
- 4- Wash with tap water.
- 5- Decolorize for 5 to 15 Sec with 95% ethyl alcohol.
- 6- Wash with tap water.
- 7- Apply Counterstain with Safranin for 1 min.
- 8- Wash with tap water
- 9- Dry the slide with paper and examine under oil immersion.

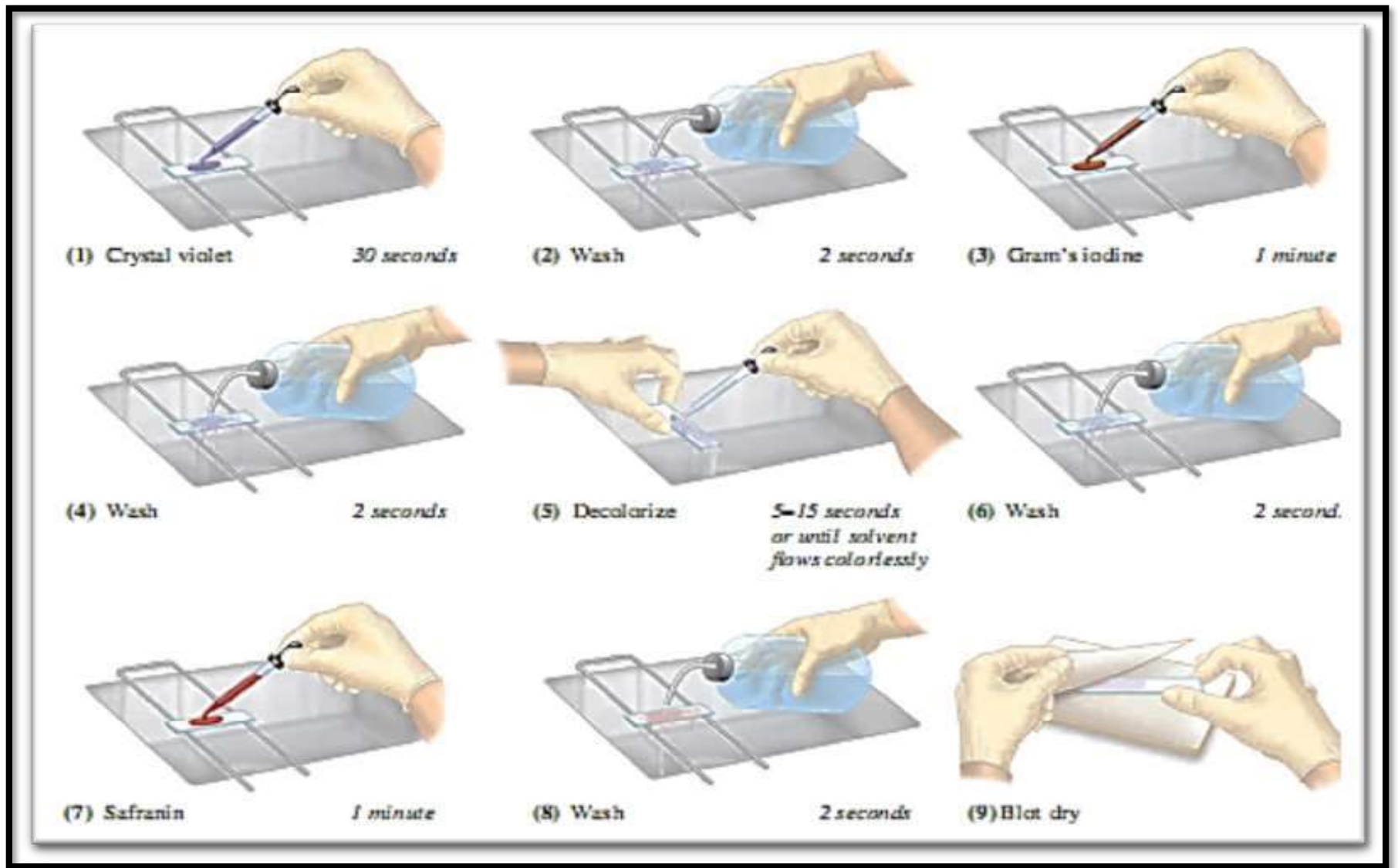


Figure (3): Gram staining procedure.

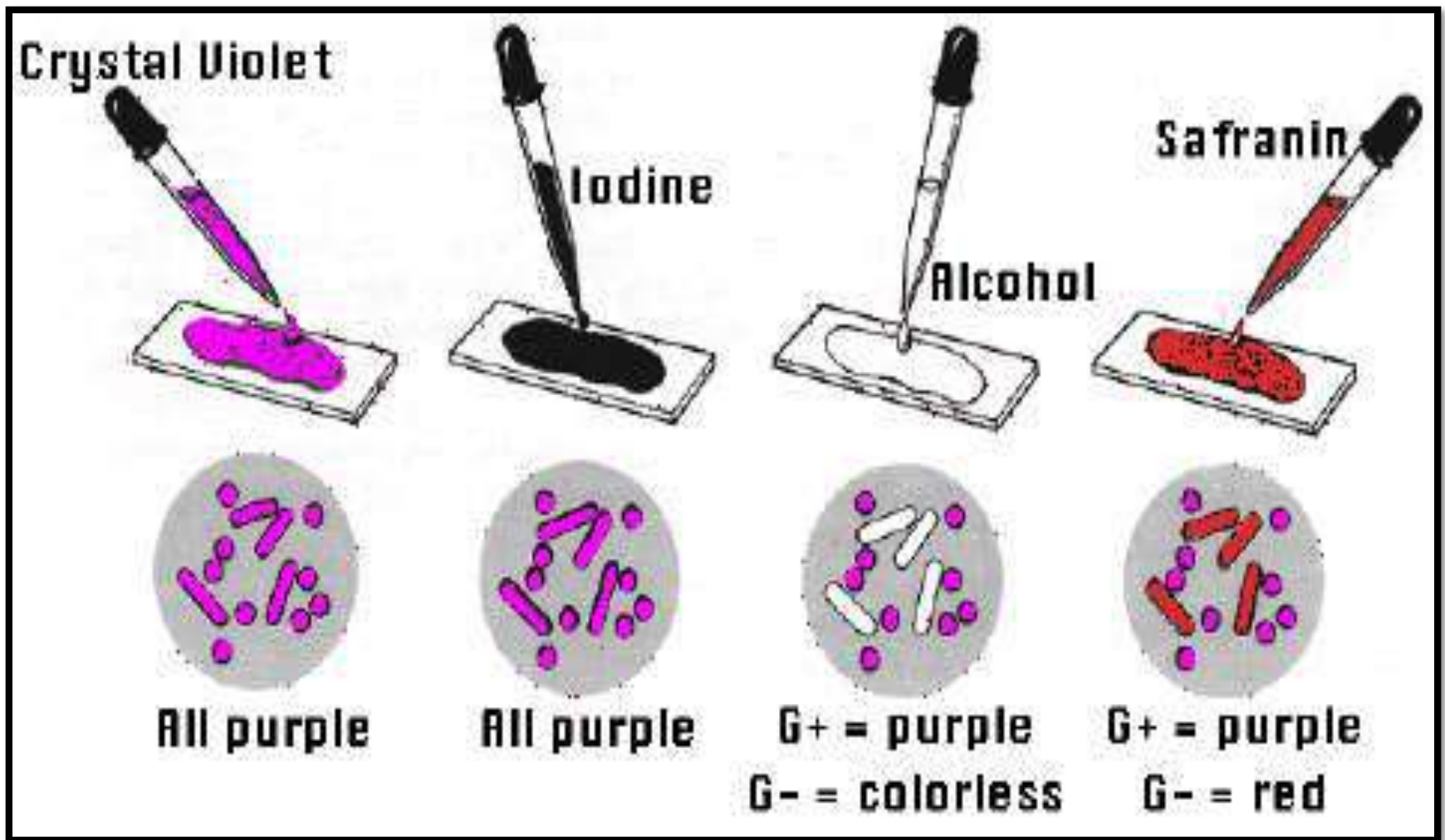


Figure (4): Gram staining procedure.

Thanks
for
Listening



Differential Stain

Diagnostic Microbiology

Lab: 6

Assist lecture: Nadia Khalid Mustafa

The Acid-Fast Stain A.F.S./ Zziehl–Neelsen Z.N./ Acid Fast Bacilli A.F.B

Is a differential stain used to identify acid-fast organisms such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

Acid-fast organisms have wax-like cell walls called mycolic acid contain:

- 1- large amounts of fatty acids.
- 2- complex lipids.
- 3- waxes.

Acid-fast organisms are highly resistant to disinfectants, dry conditions and staining by ordinary methods therefore require a special staining technique.

Acid-fast Stain reagents :

1- Carbofuchsin (Ziehl–Neelsen)(ZN): is the primary stain, act as lipid soluble, phenol is a part of this dye which helps the stain penetrate the cell wall, this is further assisted by the addition of heat.

2- Decolorizer: 3% Hydrochloric acid + 95% alcohol = (acid alcohol).

3- Counterstain: methylene blue.

Procedure:

1. **Smear** the sputum over the glass slide, and fix by heating.
2. Pour **carbol-fuschin** over smear and heat gently until fumes appear stand for 5 minutes, wash it off with **water**.
3. Pour **acid alcohol**, and wait for 1 minute, repeating this step until the slide appears light pink in color, wash off with **water**.
4. Pour **methylene blue**, wait for 2 minutes, again wash with water.
5. Allow it to air **dry** and examine under **oil immersion** lens .

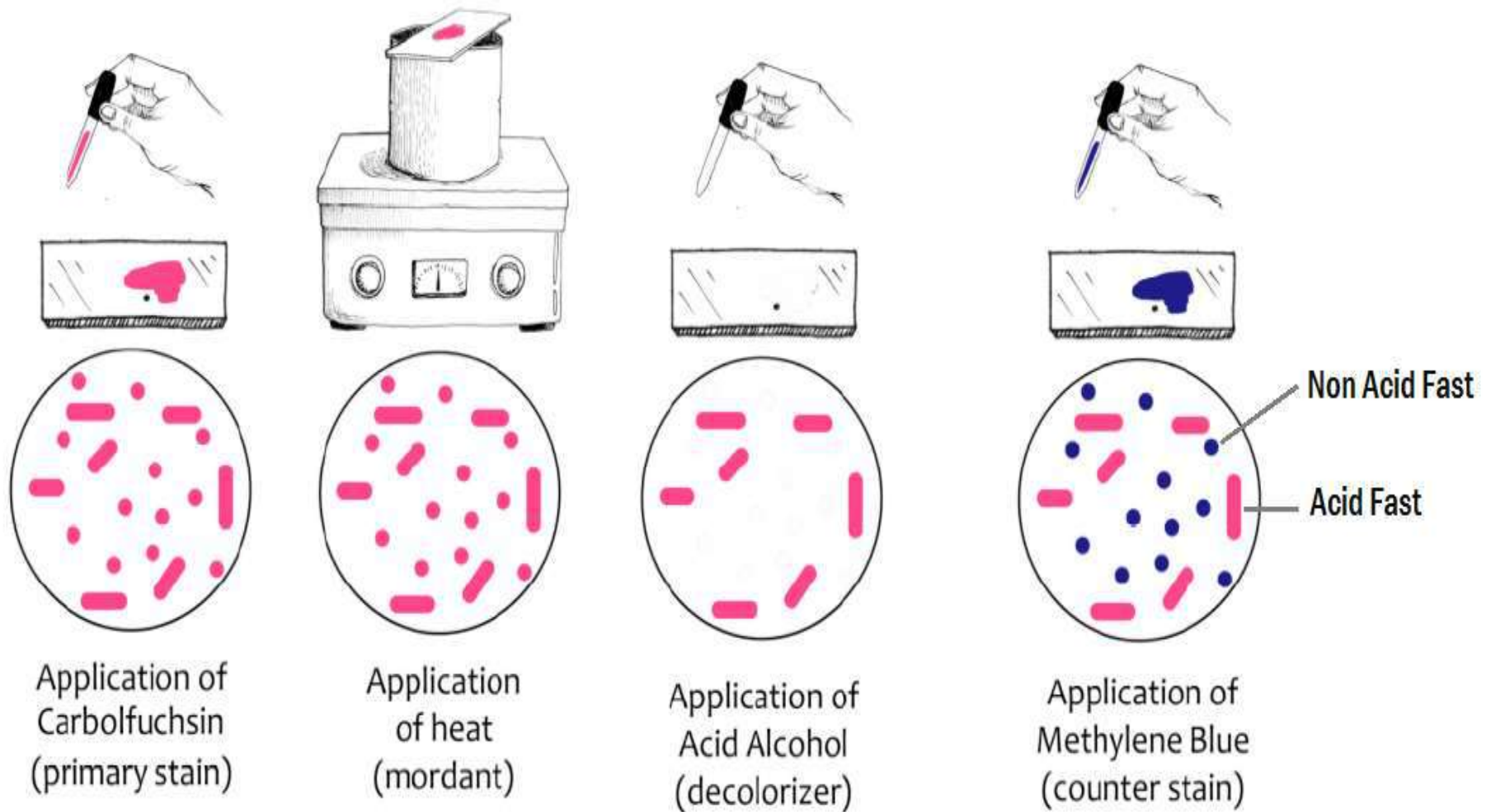


Figure (1): procedure of acid-fast stain

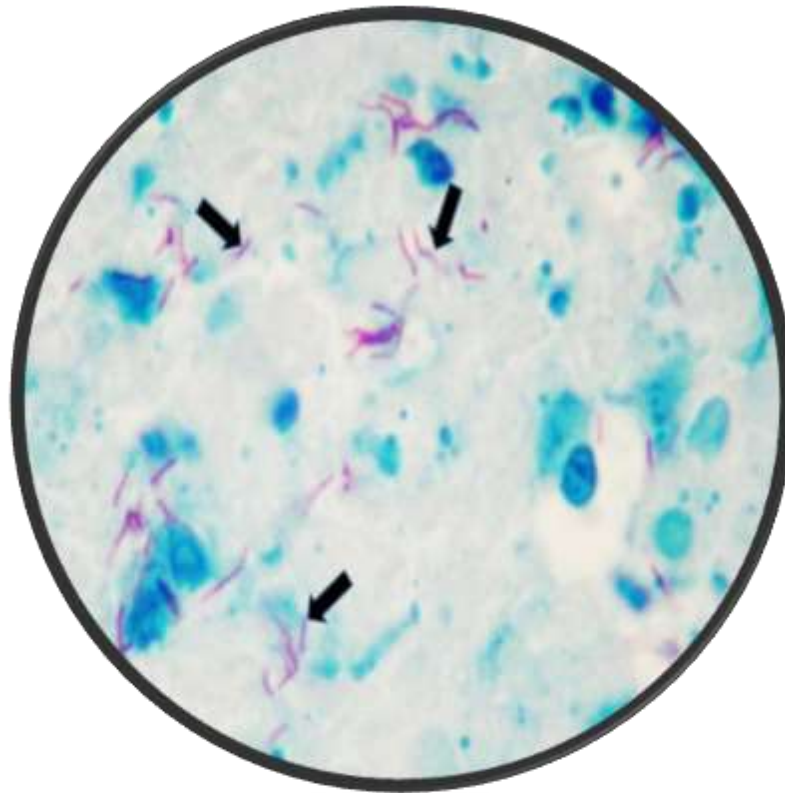


Figure (2) : Positive A.F.B.

ACRIDINE ORANGE STAIN

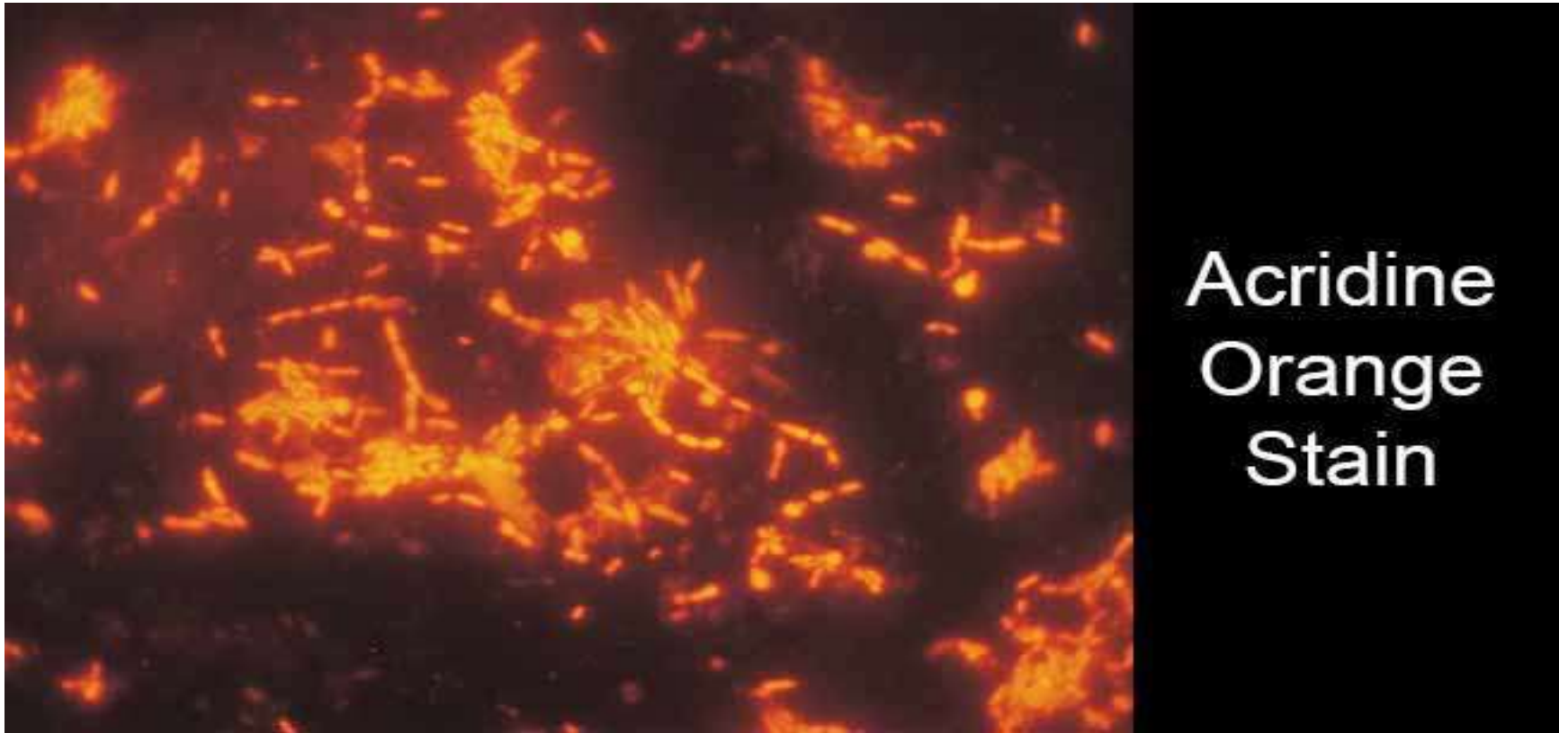
Acridine Orange Stain is used as a fluorescent staining agent to detect the presence of bacteria in blood cultures and cerebral spinal fluid C.S.F.

This stain can interfere into nucleic acid.

under UV light, bacterial and fungal nucleic acid fluoresces **orange** whereas background mammalian nucleic acid fluoresces **green** or **dark**.

Procedure:

1. The prepared slide is fixed in methanol and air-dried.
2. Flood the slide with Acridine Orange Stain for 2 Minutes.
3. Wash the slide with tap water and air dry.
4. Examine under UV light.



Acridine
Orange
Stain

Figure (3) : Positive Acridine orange stain

Fluorochrome dye / Auramine - Rhodamine staining

This dye has an affinity for the mycolic acid of the Mycobacteria,

which appear **bright yellow** or **orange** against a **greenish** or **dark** background.

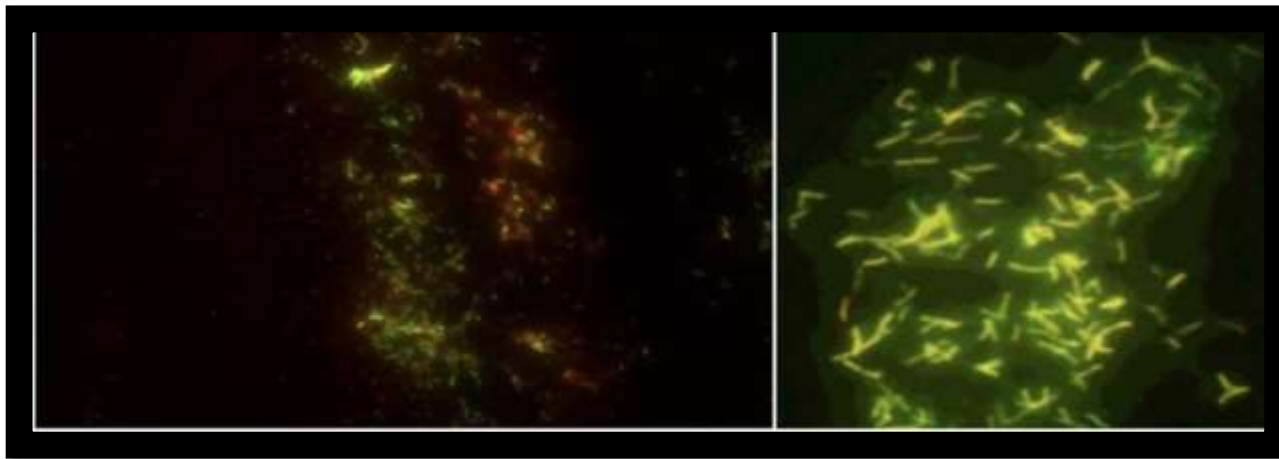


Figure (4) : Positive Auramine Rhodamin

Procedure:

1. Prepare a thin **smear** of the specimen on the slide, and gently **heat** fix the smear avoiding overheating.
2. Add **the Auramine-Rhodamine** Dyes on the smear and allow it to stand for 15 minutes. Do not apply heat.
3. Wash with the **water**.

NOTE: Ensure the water is chlorine-free water (used distilled water) because chlorine interferes with fluorescence.

4- Add the **decolorizing** agent (acid-alcohol) for 2-3 minutes, **wash** with distilled water and remove excess water by shaking the slide.

5-Flood the smears with **potassium permanganate (counterstain)** for 2 minutes exactly, **Wash** with distilled water and allow to air dry.

6- Examine under a **fluorescent** microscope.

Fungal stain

Lactophenol cotton blue (LCB)

The lactophenol cotton blue solution acts as a mounting solution as well as a staining agent.

The solution is blue in color and it is made up of a combination of three main reagents:

- 1- Lactophenol:** a solution of (Phenol: It acts as a disinfectant by killing any living organisms).
- 2- Lactic acid:** to preserve the fungal structures.
- 3- Cotton blue:** to stain or give color to the chitin on the fungal cell wall and other fungal structures.

Procedure:

- 1- Wearing gloves.
- 2- Clean skin surface with alcohol 70%.
- 3- scrap a small quantity of material to be examined (skin, nail, hair) on a microscope slide.
- 4- add a drop of 10% KOH.
- 5- Gently pass the slide through a flame of a burner.
- 6- add one or two drops of L.C.B. solution.
- 7- Place a cover slip over the specimens, and examine microscopically for the presence of spores and hyphae.

Note :

the nails may have to be kept in KOH for 30 minutes before they become soft .

Hairs clip by forceps and avoid cutting the hair.

Results:

Positive: Fungal element seen (spores and hyphae)
or Fungal element seen (spores only),
or Fungal element seen (hyphae only).

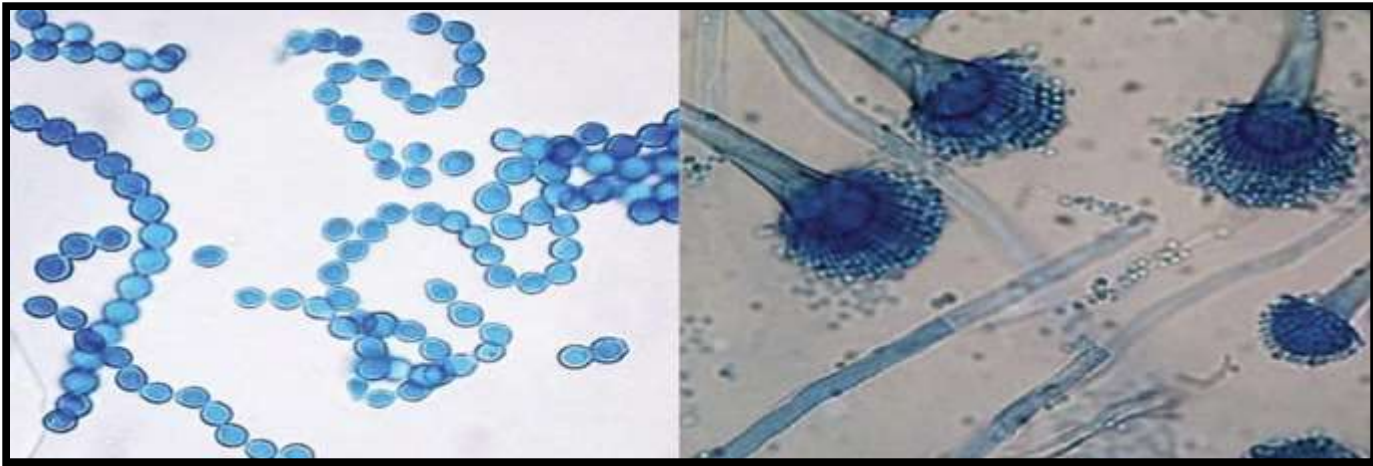


Figure (5) : Positive fungi

Negative: No fungal element seen.

Thanks
for
Listening

كلية النور الجامعة

قسم تقنيات المختبرات الطبية

Diagnostic Microbiology

المرحلة الرابعة

Lab 7 /2023



Culture Media

Diagnostic Microbiology

Lab: 7

Assist lecture:Nadia Khalid Mustafa

Culture media: is defined as a **solid** or **liquid** or **semi solid** preparation used for the growth , transport and storage of microorganisms.

Any culture media must contains all the nutrients required for the growth of microorganisms such as: (carbohydrates, nitrogen, carbon, phosphorus, sulfur and various minerals (Ca., Mg., Na.) and water .

Classification of culture media based on density:

1-Solid media :

Contains agar at a concentration of 2%, is useful for:

- ▶ isolation bacteria.
- ▶ or for determining (morphology ,pigmentation , hemolysis).

Colony: visible growth of bacteria on the surface of solid medium arising from a single cell.

2-Semi solid media :

They are prepared with agar at concentrations of 0.5%, is useful for:

- ▶ cultivation of microaerophilic bacteria.
- ▶ or for determination of bacteria motility .

3-Liquid (broth) media:

These media without solidifying agents, is useful for:

- ▶ propagation of large number of organisms and fermentation studies.

Classification based the functional use:

1-general purpose media / basic media :

Simple media that supports most bacteria , these media are used for primary isolation of microorganism.

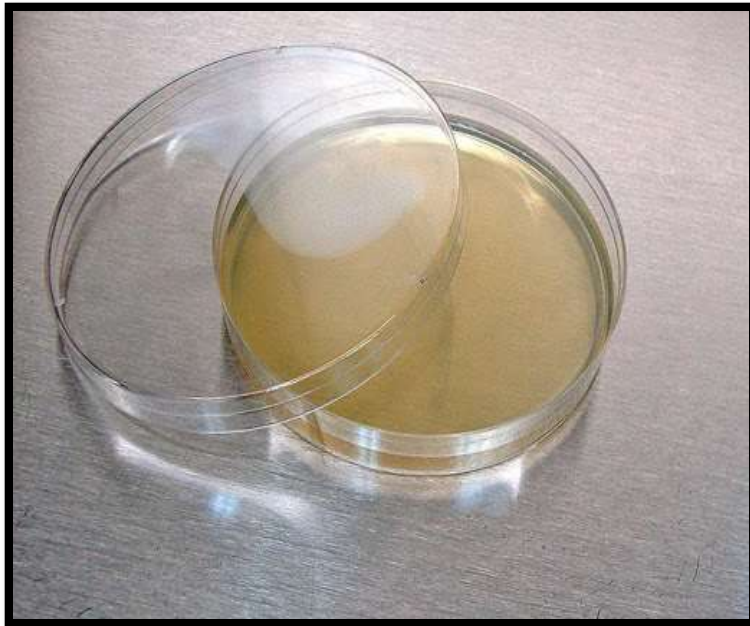


Figure (1): Nutrient agar (N.A.)



Figure (2): Nutrient broth (N. B.)

2- Enriched media :

Addition of extra nutrients in the form of blood, serum and egg yolk .

used to grow (fastidious bacteria). Ex:

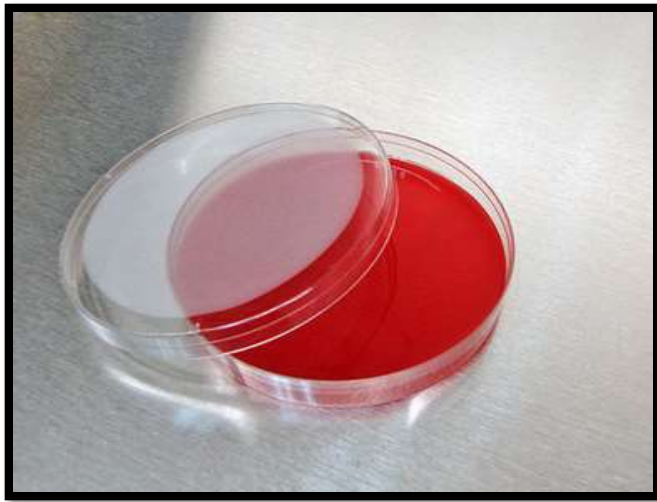


Figure (3): Blood agar (B. A.)



Figure (4): Chocolate agar (CBA)

Chocolate Blood Agar (CBA): It is the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80°C.

Chocolate agar is used for:

growing fastidious bacteria, such as:

Haemophilus influenza and *Neisseria meningitidis*.

In addition, some of these bacteria, most notably *H. influenzae* , need growth factors such as

nicotinamide adenine dinucleotide (factor V or NAD) and hemin (factor X), which are inside red blood cells; thus, a prerequisite to growth for these bacteria is the presence of red blood cell lysates.

NOTE: The agar is named for its color and contains no chocolate products.

3- Selective media :

- ▶ this is a type of medium in which an inhibitory substance is added to favor the growth of wanted bacteria.
- ▶ Inhibitory substance may be dyes ,bile salts , alcohol ,acids and antibiotics .
- ▶ This medium are used to isolate a particular bacteria from a specimens.

Ex:

- ▶ Thayer martin agar: used to recover *Neisseria gonorrhoeae* contains vancomycin ,colistin.
- ▶ Mannitol salt agar: used to recover staphylococcus genus . High salt concentration (10%NaCL) inhibits most bacteria .
- ▶ MacConkey agar:_used for enterobacteriaceae members contains bile salt and crystal violet that inhibits most gram positive bacteria.

4- Differential media :

These media contain an indicator which changes the color when a bacterium grows in them .

Ex:

A- Blood agar:

Used for the growth of types of bacteria that need proteins and vitamins present in the blood. It is prepared by adding blood to the nutrient agar medium after sterilization and cooling to a temperature of 50 °C.

Blood agar medium they are considered the rich and differentiation medium at the same time, in addition to being a rich medium, it distinguishes between types of pathogenic bacteria depending on their ability to analyze blood by secreting an enzyme called Hemolysin.

according to hemolytic activity of bacteria , there are three type of hemolysis :

A- Alpha-hemolysis: is partial hemolysis. Colonies are surrounded by a green , opaque zone ----- *Streptococcus pneumoniae*.

B- Beta-hemolysis: is complete hemolysis it is characterized by a clear zone surrounding the colonies ----
- *Staphylococcus aureus*. , *Pseudomonas aeruginosa*.

C- Gamma-hemolysis: no hemolysis. There are no zones around the colonies ----- *Staph. epidermidis* , *Klebsiella pneumoniae*.

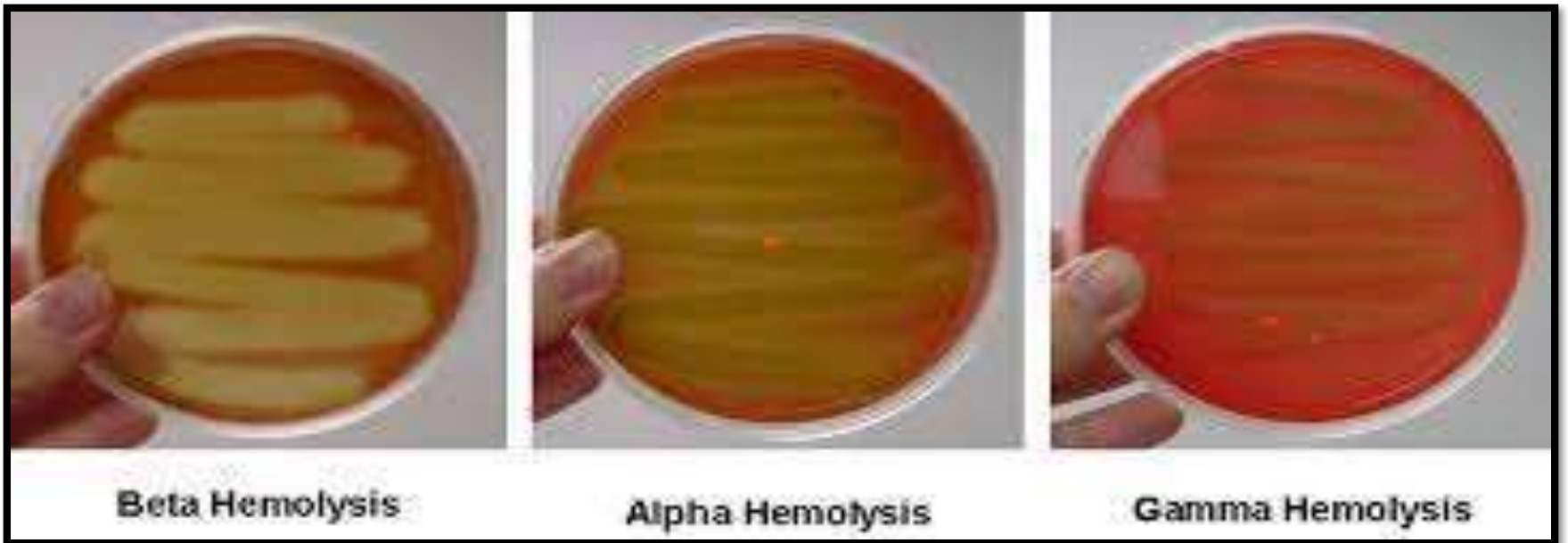
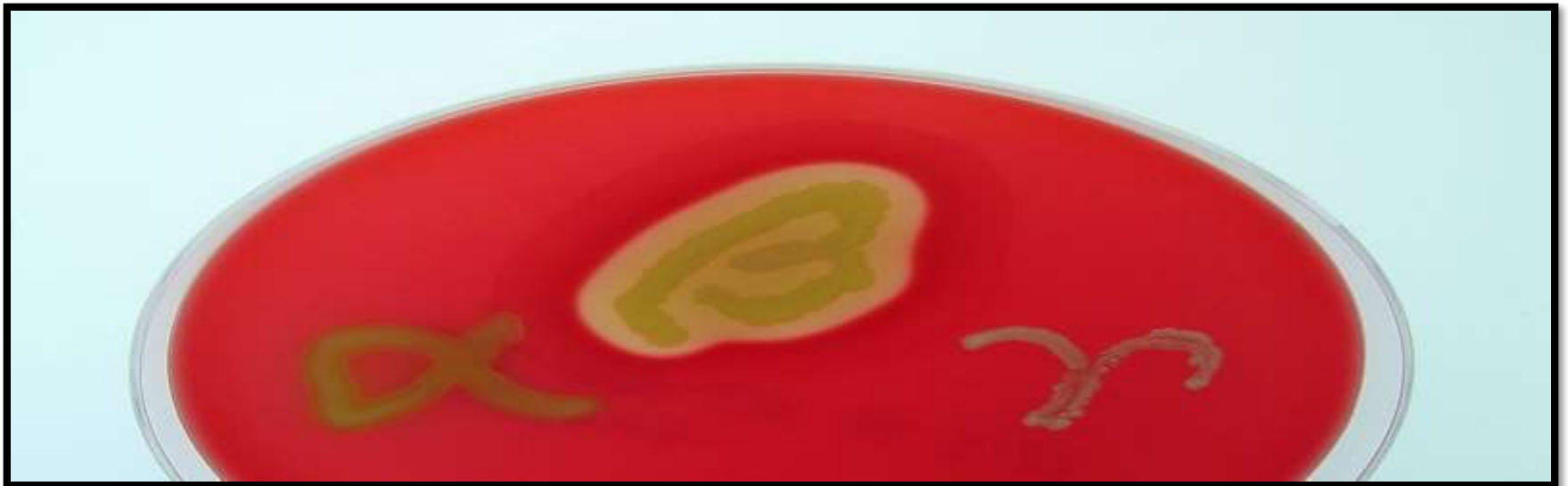


Figure (5): type of hemolysis

B- Mannitol salt agar:

Is a selective and differential medium.

Differentiate between staphylococcus by sugar mannitol fermentation.

Mannitol fermentation produce acids that change the medium pH.

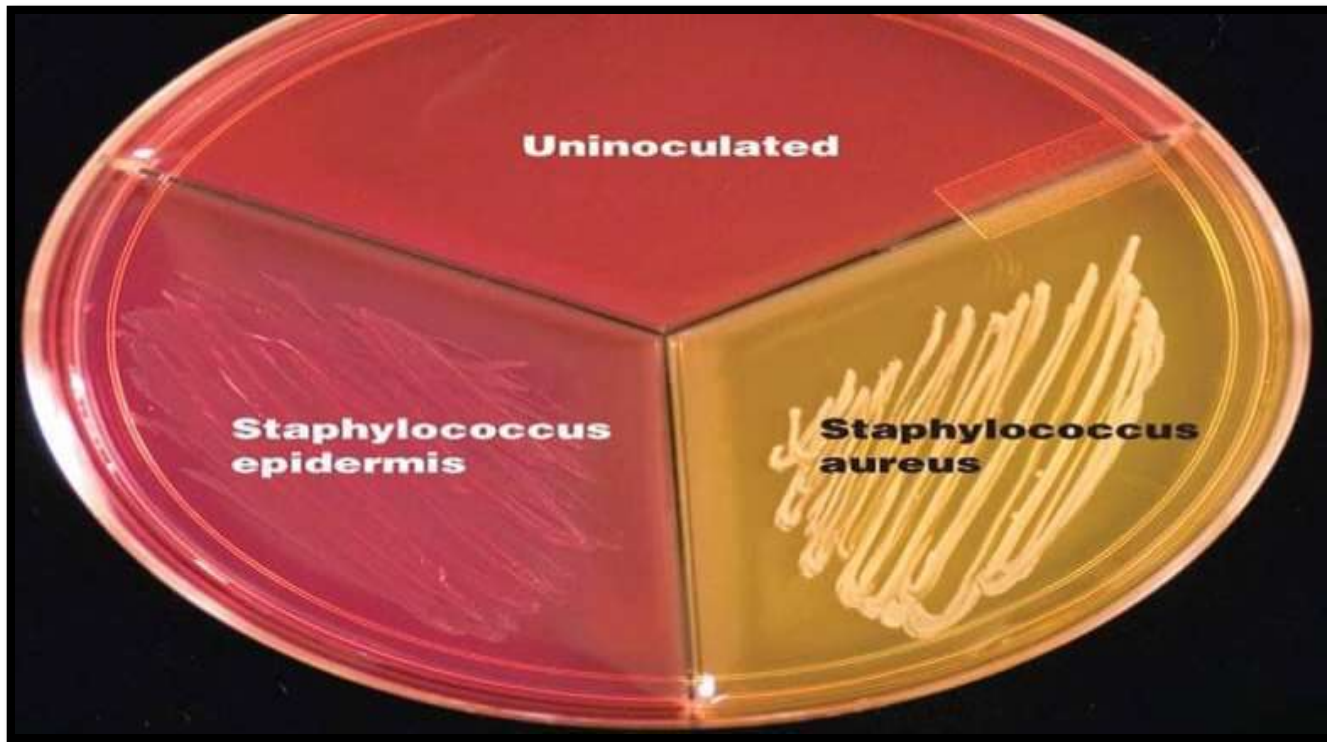


Figure (6): Mannitol salt agar

C- MacConkey agar:

is a selective and differential media

Differentiate between gram negative bacteria by their ability to ferment lactose.



Figure (7): MacConkey agar

D- Eosin Methylene Blue agar (EMB):

Is a selective and differential medium used to isolate enterobacteriaceae Eosin and methylene blue are ph indicator dyes and inhibit Gm (+) bacteria.



Figure (8): *E.coli* on EMB

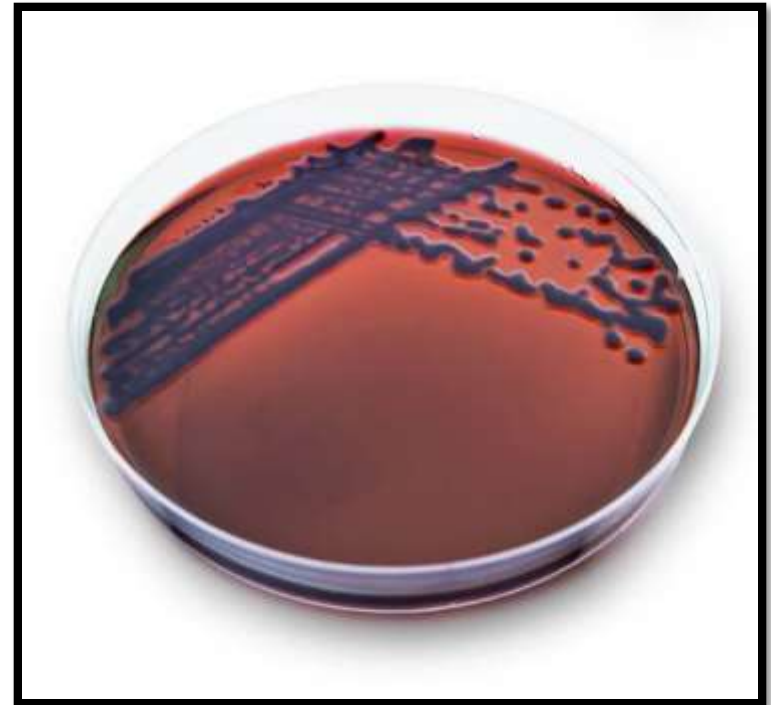


Figure (9): *Klebsiella pneumoniae* on EMB

5- Transport media :

These are used for the temporary storage of specimens being transported to the laboratory for cultivation.

characteristics of transport media:

1. It should be non-toxic.
2. It should inhibit the bacterial growth.
3. It should be easy to carry and transport.



Figure (10): Transport media

6- anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content reduced oxidation .

Ex:



Figure (11): Thioglycollate broth

Thanks
for
Listening

كلية النور الجامعة

قسم تقنيات المختبرات الطبية

Diagnostic Microbiology

المرحلة الرابعة

Lab 8 /2023



Biochemical Tests of Microorganisms

Part One

Diagnostic Microbiology

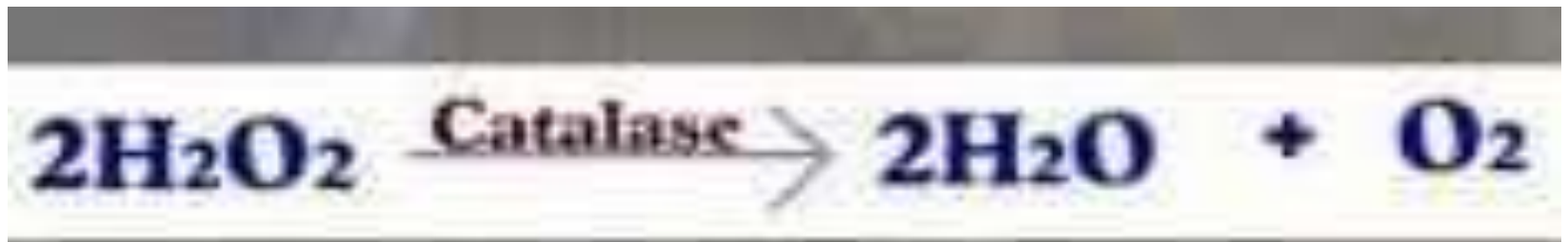
Lab: 8

Assist lecture: Nadia Khalid Mustafa

because there are many species of bacteria having similar shape and size, so we need biochemical tests that used for the identification of bacteria species based on the differences in the biochemical activities.

1-Catalase Test:

This test is used to identify organisms that produce the enzyme **catalase** breaking **hydrogen peroxide** into **water** and **oxygen** gas. The bubbles resulting from production of oxygen gas clearly indicate a positive result.



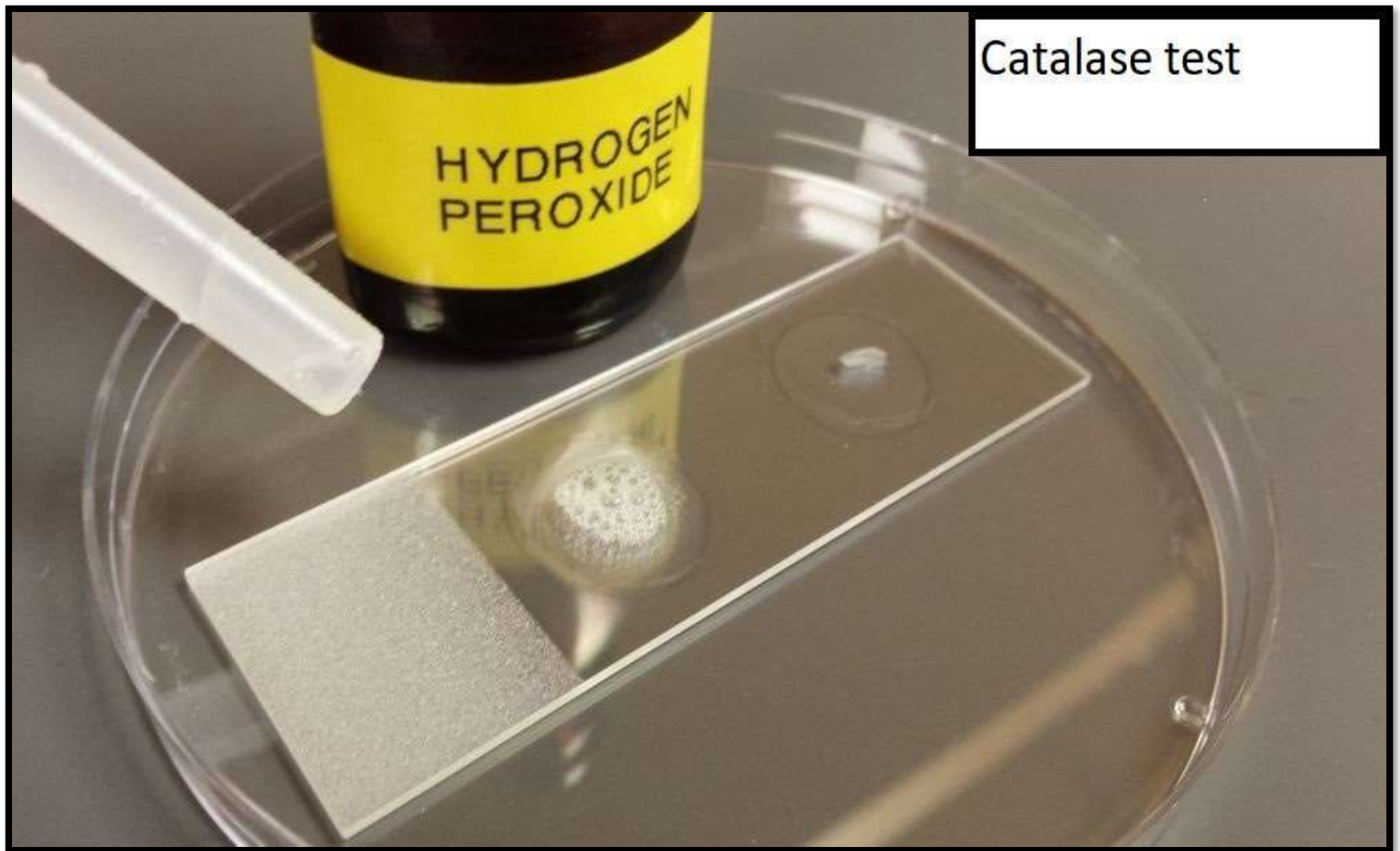
Procedure:

The catalase test is done by:

- 1- transfer bacterial colony by wooden stick on the slide,
- 2- then add a drop of hydrogen peroxide on them.
- 3- Note whether or not bubbles form.

The Staphylococcus ---- Positive.

The Streptococcus ---- Negative.



Catalase test

Figure (1): Catalase test

2- Indole test:

Is used to determine the ability of the organism to convert tryptophan into indole, pyruvic acid and ammonia by **tryptophanase enzyme**.

The presence of indole is detected by addition of Kovac's reagent,

Kovacs' reagent reacts with the indole, producing a bright red compound on the surface of the medium

Tryptophane
amino acids

Tryptophanase

→ **Indole + Pyruvic acid + NH₃**

↓ **Kovac's Reagent**

Red color in upper organic layer'

Procedure:

- 1- Inoculate peptone water (is a rich in tryptophan) with young bacterial culture.
2. Incubate at 37 °C for 24_48 hours.
- 3- Add 5 drop of kovec's reagent to the broth culture.
- 4- Observe for the presence or absence of red ring.

Positive ----- *E. coli*.

Negative ----- *Pseudo. aeruginosa*.

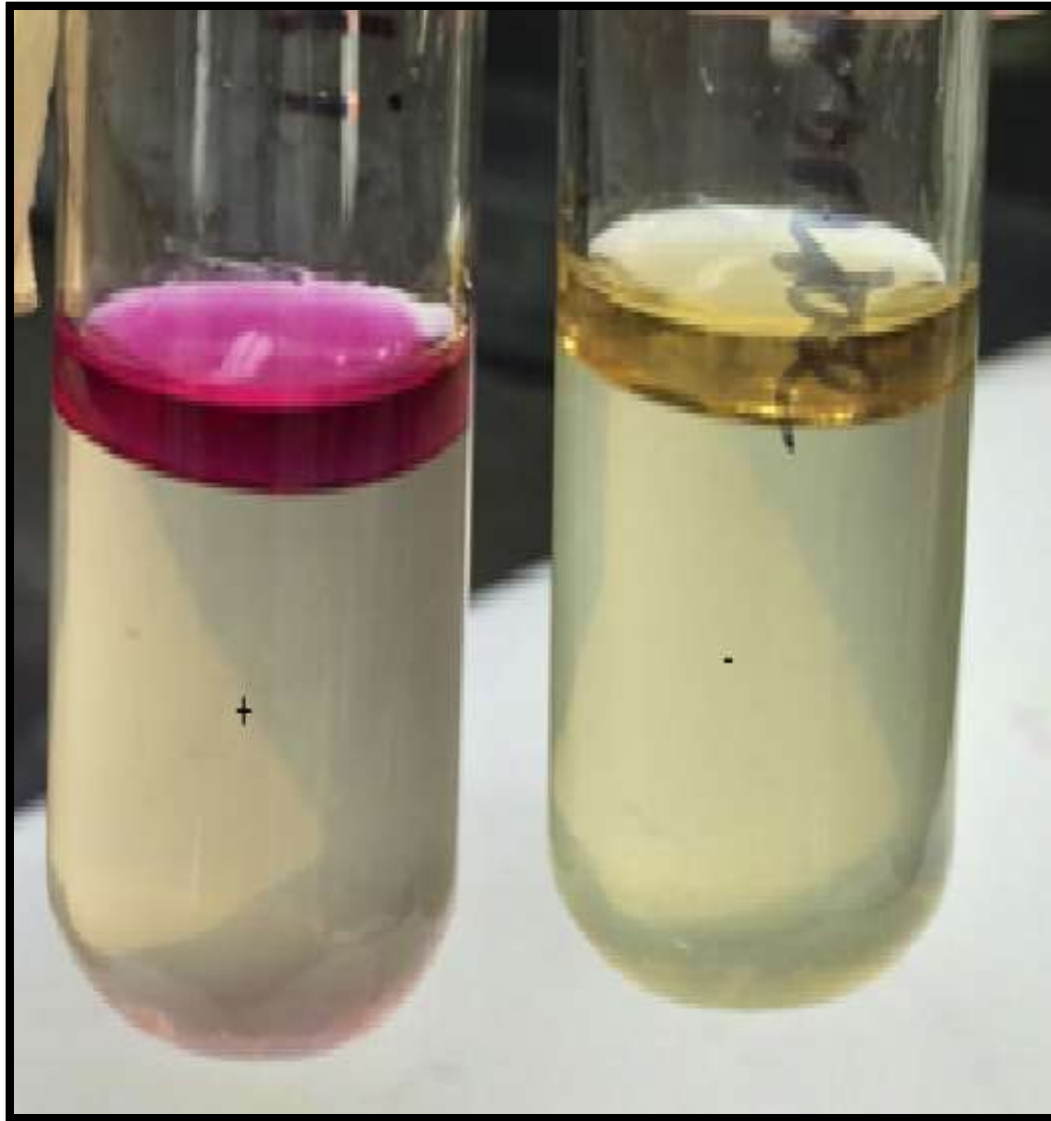


Figure (2): Indole test

Thanks
for
Listening

كلية النور الجامعة

قسم تقنيات المختبرات الطبية

Diagnostic Microbiology

المرحلة الرابعة

Lab 9 /2023



Biochemical Tests of Microorganisms

Part Two

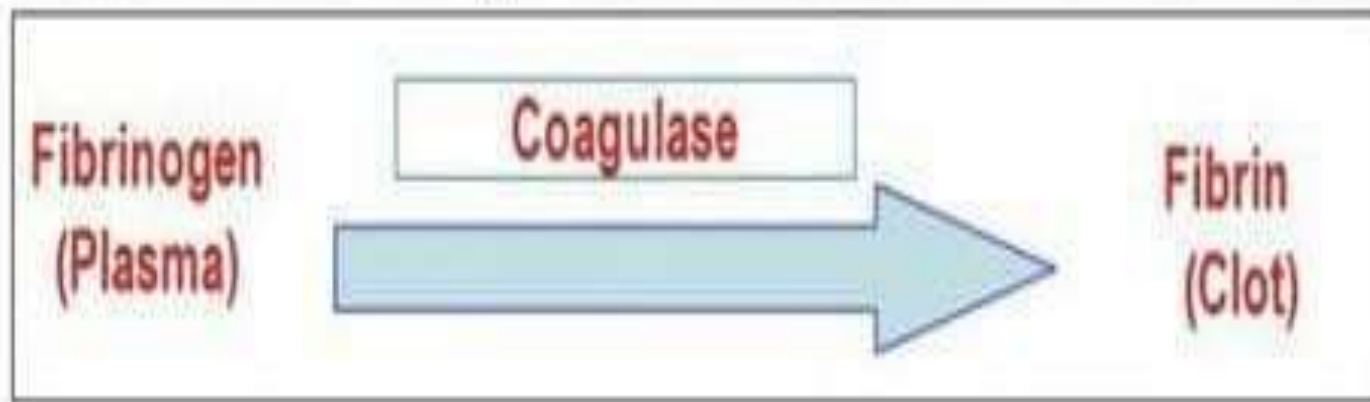
Diagnostic Microbiology

Lab: 9

Assist lecture: Nadia Khalid Mustafa

1-Coagulase test:

Coagulase is an enzyme that clots blood plasma.



Procedure:

This test is done by **tube coagulase method** or **slide coagulase method**:

- 1- Place a drop of distilled water on a glass slide.
- 2- With a wooden stick, transfer a colony of bacteria and mix it with a drop of water.
- 3- Add a drop of blood plasma to the suspension and mix well.

Agglutination ----- Positive like *Staph. aureus*.

No agglutination ----- Negative like Other Staphylococcus.



Figure (1): slide coagulase method



Figure (2): tube coagulase method

2- Methyl red test (MR):

This test is used to determine :

The ability of bacteria to **oxidize glucose** and produce high concentrations of **acidic** end products,

the acid **decreases the PH to 4.5 or below,**

which is indicated by a change in the color of **methyl red** from **yellow** to **red**.

Procedure:

1- Incubate glucose phosphate peptone water media (MR-VP) with young bacterial culture for 24- 48 h at 37°C.

2- Add 2-3 drops methyl red reagent into the broth.

Red color solution : Positive ----- *E. coli*

Yellow: Negative ----- Klebsiella.

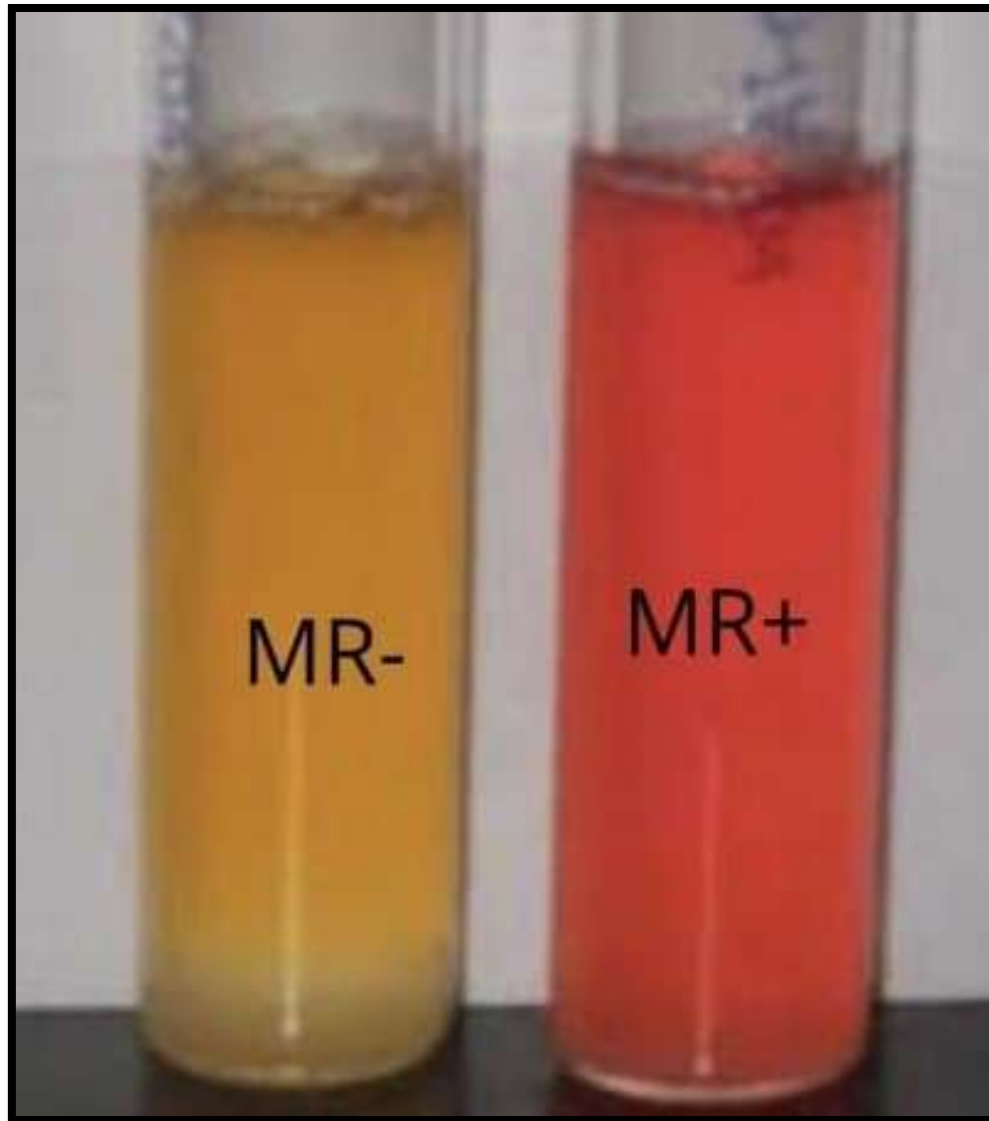


Figure (3): Methyl red test

Thanks
for
Listening

كلية النور الجامعة
قسم تقنيات المختبرات الطبية
Diagnostic Microbiology
المرحلة الرابعة
Lab 10 /2023



Biochemical Tests of Microorganisms

Part Three

Diagnostic Microbiology

Lab: 10

Assist lecture: Nadia Khalid Mustafa

1- Oxidase Test:

This test is used to identify microorganisms containing the enzyme **cytochrome oxidase**.

Procedure:

- 1- Wet filter paper with the indicator composed of N,N,N,N tetramethyl- p-phenylenediamine dihydrochloride (TMPD).
- 2- Use a wooden stick to transfer a large mass of pure bacteria to the filter paper.
- 3- Observe the filter membrane for up to three minutes. If the area of inoculation turns dark-blue the result is positive. If a color change does not occur the result is negative.

Positive ----- Pseudomadaceae.

Negative ----- Enterobacteriaceae.

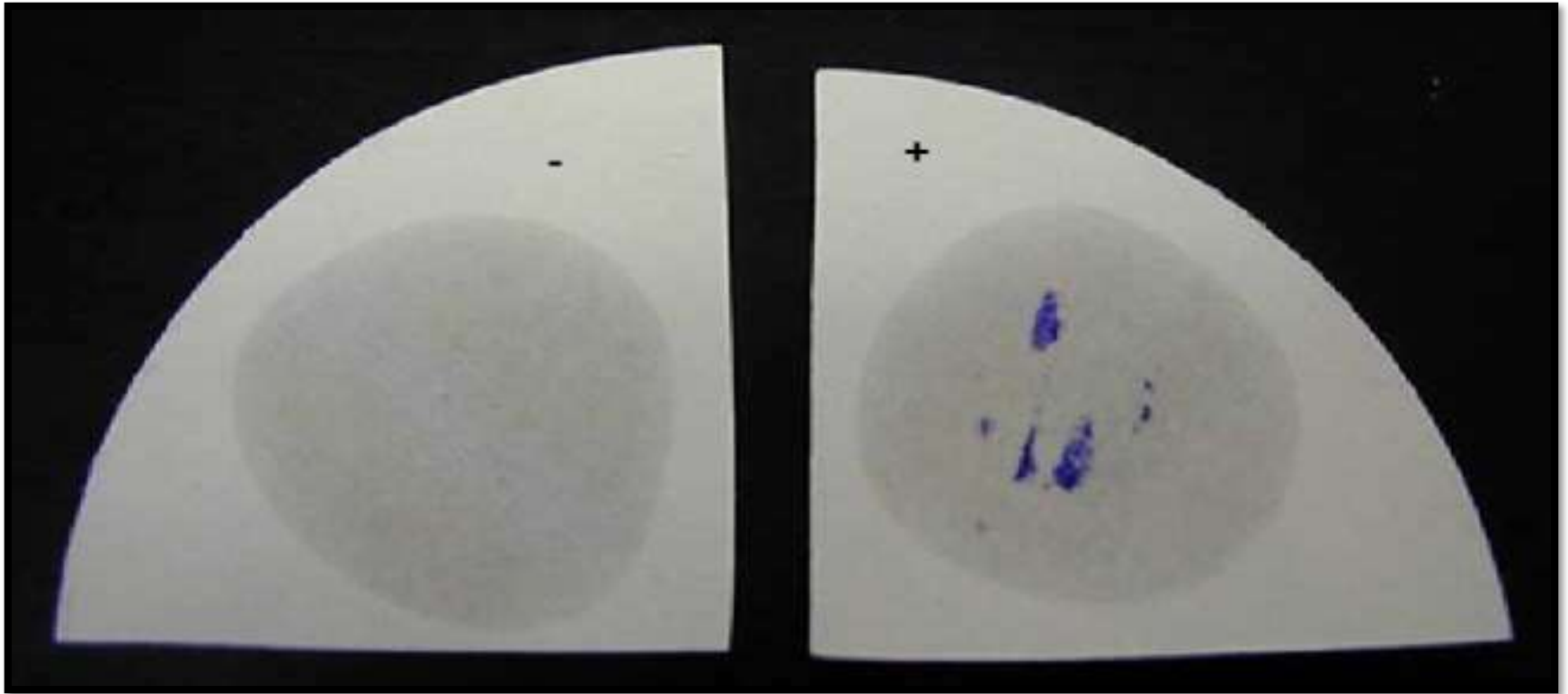


Figure (1): Oxidase test

2- Urease test:

This test is used to detect if the bacteria possess the **enzyme Urease** that hydrolyzing **urea** into **ammonia** and **CO₂** .

Procedure:

1. Inoculate the urea agar with the required bacteria.
2. Incubate at 37°C for 24 - 48 hrs.

Positive Pink color ----- **Klebsiella, Proteus,**
Helicobacter pylori.

Negative Yellow color ----- *E.coli*



Figure (2): Urease test

Thanks
for
Listening

كلية النور الجامعة

قسم تقنيات المختبرات الطبية

Diagnostic Microbiology

المرحلة الرابعة

Lab 11 /2023



Biochemical Tests of Microorganisms

Part Four

Diagnostic Microbiology

Lab: 11

Assist lecture: Nadia Khalid Mustafa

1- Citrate test:

Purpose of the test: To detect bacteria capable of consuming citrate as a source of carbon and ammonia as a source of nitrogen.

Bacteria that grow on this medium produce an enzyme citrate permease.

Media : Simmons Citrate agar.

Reagent: Bromothymol Blue indicator.

Note: The reagent is included in the medium.

The Procedure:

1. The slant is streaked only with the required bacteria.
2. Incubate at 37 °C for 48 hrs.
3. Observed a color change.

Negative ----- Green like *E. coli*.

Positive ----- Blue like *Pseudomonas spp.*

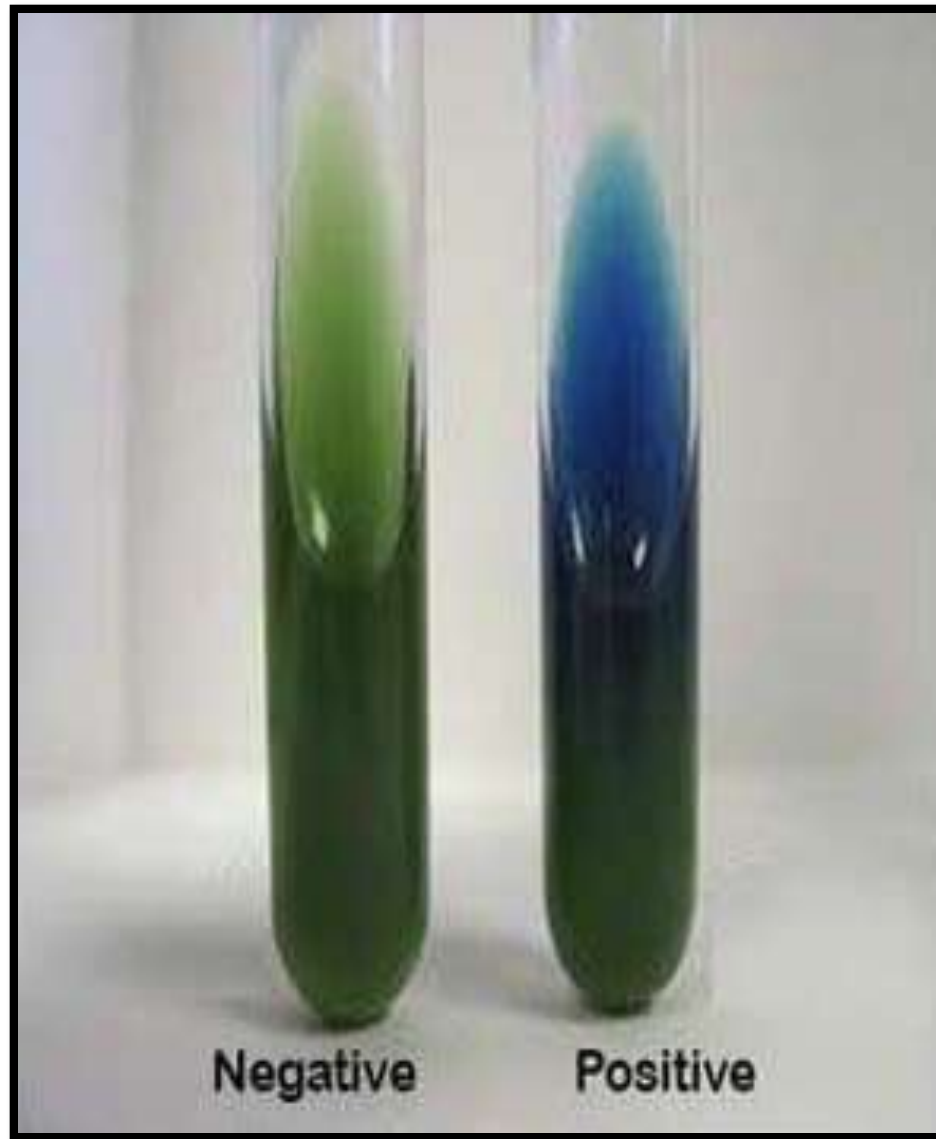


Figure (1) : Citrate test.

2- Triple Sugar Iron Agar test (TSI):

The name of the test indicates the name of the medium containing the 3 types of sugars: **glucose, sucrose and lactose**. In addition to **ferrous sulfate** compound and **red phenol** reagent.

This test is used to detect:

A- Fermentation of sugars.

B- Detection of gases CO_2 .

C- Detection of H_2S .

Interpretation of results:

- If **neither lactose/sucrose nor glucose** is fermented, both the **butt and the slant will be red.** (K / K), Like: *Pseudomonas spp.*
- If the **glucose** is fermented, the **butt will be yellow.** but the **slant will be red.** (K \ A), Like: *Salmonella typhi.*
- If **lactose and sucrose** is fermented, the **butt and slant will be yellow.** (A \ A), Like: *E.coli.*

- if **H₂S** is produced, the **black** color of ferrous sulfide is seen. Like *Proteus spp.*



- Some organisms generate **gases** CO₂, which produces **bubbles or cracks** on the medium.

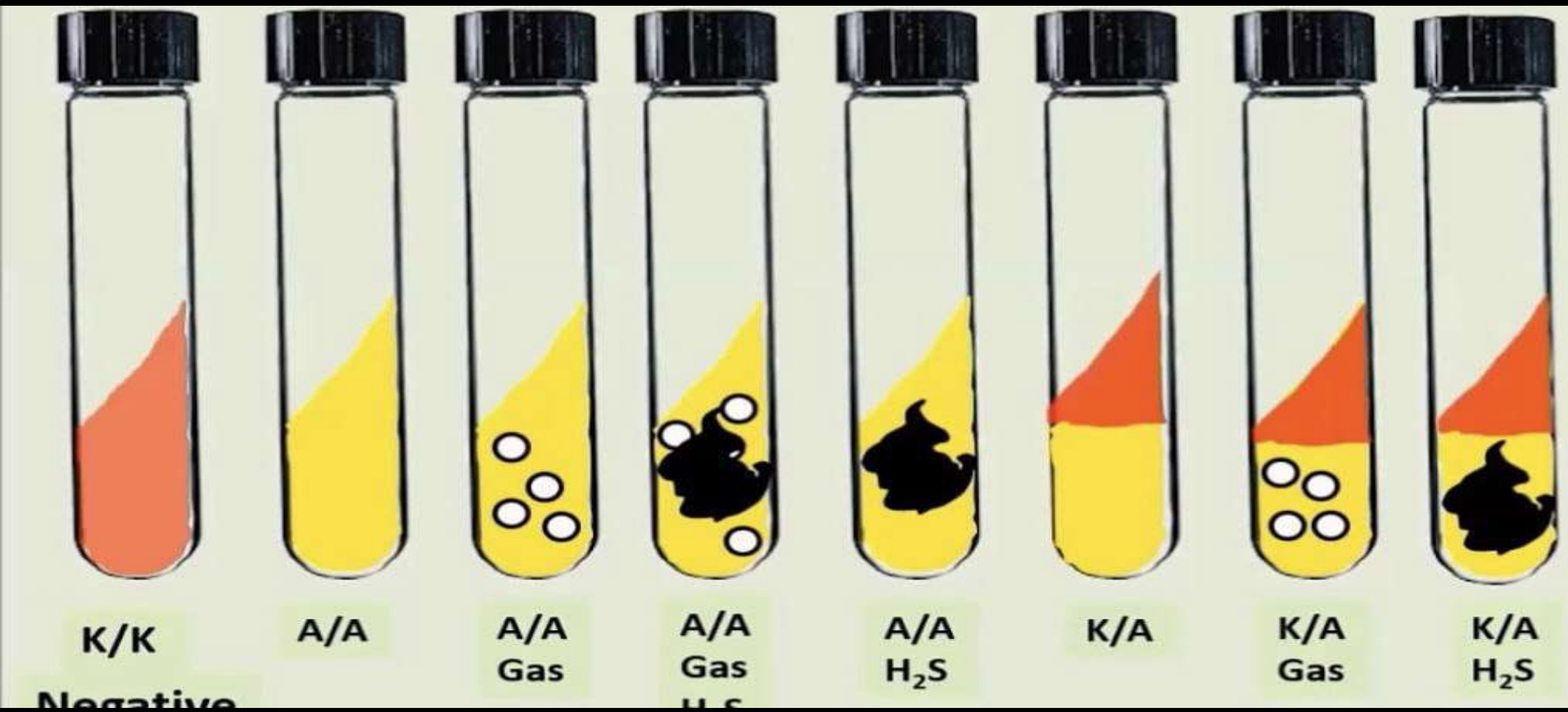


Figure (2): TSI test

A : Acidic.

K: Alkaline.

Note: When reading the result, we read the slant, then the bottom

(Slant / butt Gas^(+ or -) H₂S^(+ or -))

Thanks
for
Listening

كلية النور الجامعة

قسم تقنيات المختبرات الطبية

Diagnostic Microbiology

المرحلة الرابعة

Lab 12 /2023



Analytical Profile Index (API 20E Test)

Diagnostic Microbiology

Lab: 12

Assist lecture: Nadia Khalid Mustafa

API 20E Test

This is the standardized minimal translation method for the traditional biochemical group of tests, used to:

- 1- rapid identification of Enterobacteriaceae.
- 2- and other gram-negative bacteria.

*About 100 types of gram-negative bacilli can be diagnosed using this system.

*produces **21 biochemical tests**.

*The kits include strips that contain up to **20 chambers miniature biochemical tests** which are all quick, safe and easy to perform.

Note: An oxidase test is performed separately and constitutes the 21st test.

The Test Kit:

The test kit enables the following tests:

1. O-nitrophenyl-b-D-galactopyranoside (ONPG): ONPG degradation by producing the enzyme B-galactosidase, producing yellow orthonitrophenol from a colorless ONPG.

2. Arginine dihydrolase (ADH): This enzyme convert arginine to ornithine, ammonia and carbon dioxide, thus the PH increases and the color of the index changes from yellow to red.

3. Lysine decarboxylase (LDC): This enzyme turns lysine into cadaverine, which raise the PH and change the color from yellow to red.

4. Ornithine decarboxylase (ODC): This enzyme converts ornithine to putrescine, which raise the PH and change the color from yellow to **red**.

5. Citrate (CIT): utilization of citrate as a source of carbon results in a change in the PH and a change in the color of from **green** to **blue**.

6. H₂S production: which reacts with iron to form a **black** precipitate.

7. URE: test for the enzyme urease Which consumes urea to produce ammonia and changes the color from yellow to **red**.

8. Tryptophan deaminase (TDA): Which consumes tryptophan to produce indole and pyruvic acid, and in the presence of iron chloride, a **red-brown** color is formed.

9. Indole Test (IND): production of indole from tryptophan by the enzyme tryptophanase . Indole is detected by addition of Kovac's reagent To make a **pink** color.

10. the Voges-Proskauer test (VP): for the detection of acetone produced by fermentation of glucose by bacteria.

11.GEL: test for the production of the enzyme gelatinase which liquefies gelatin produce **black** color dye spread through the chamber.

12. Consumption of carbohydrates: such as glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), raminose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY), and arabinose (ARA), producing acid that changes the color of the index from blue to yellow.

API 20 E after incubation...Positive results for all tests :



API 20 E after incubation...Negative results for all tests :



Figure (1) : API 20E system

Procedure:

1. Confirm the culture is of an Enterobacteriaceae. to test this, a quick oxidase test may be performed.
2. Put some drops (5ml) of distilled water in the tray of the API test.
3. Pick a single isolated colony (from a pure culture) and make a suspension of it in 5ml of normal saline.
4. Using a pasteur pipette, fill up the compartments with the bacterial suspension.

5. Add sterile oil into the ADH, LDC, ODC, H₂S and URE compartments to provide anaerobic conditions. (a font under a name the test in strip).

6. Fully fill tubes for CIT, VP and GEL tests.

7. Close the tray and Incubate at 37°C for 18 to 24 hours.

Result Interpretation:

1- For some of the compartments, the color change can be read straight way after 24 hours, but for some reagents must be added to them before interpretation.

2- Add following reagents to these specific compartments:

- TDA: Put one drop of Ferric Chloride.
- IND: Put one drop of Kovacs reagent.
- VP: Put one drop of 40 % KOH (VP reagent 1) and One drop of α -Naphthol (VP Reagent 2).

3- Positive and negative results are recorded on a specific sheet provided by manufacturer company, as in figure 2, with corresponding values given (1, 2 or 4) for the positive test and the value (0) for the negative test.

4- Collect the value of all three tests to obtain a final number (code) consisting of seven numbers.

5- Compare the resulting final number with the analytical profile index catalog or api web (online), which gives the full scientific name for bacterial isolation.

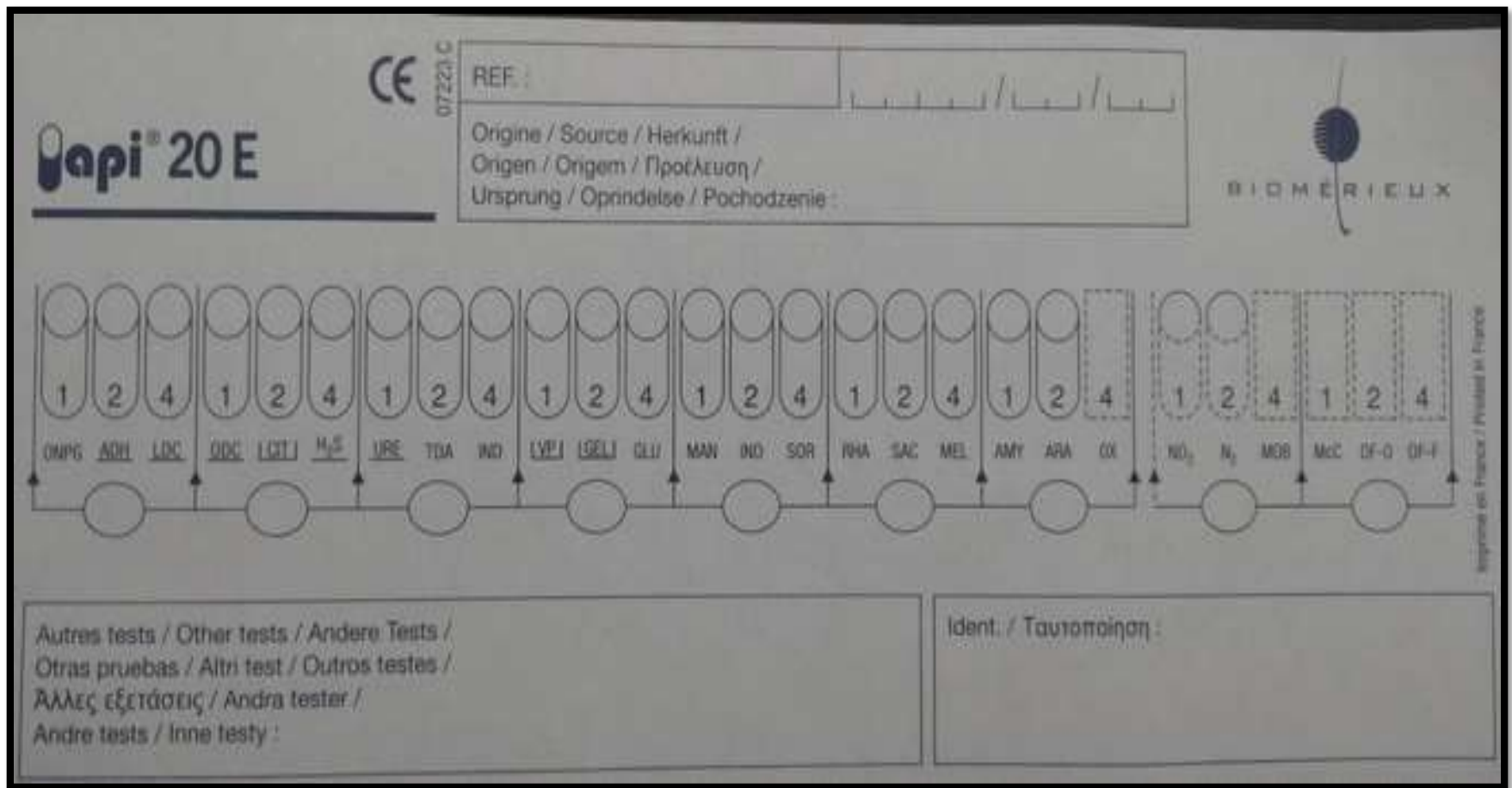


Figure (2) : API 20E result sheet

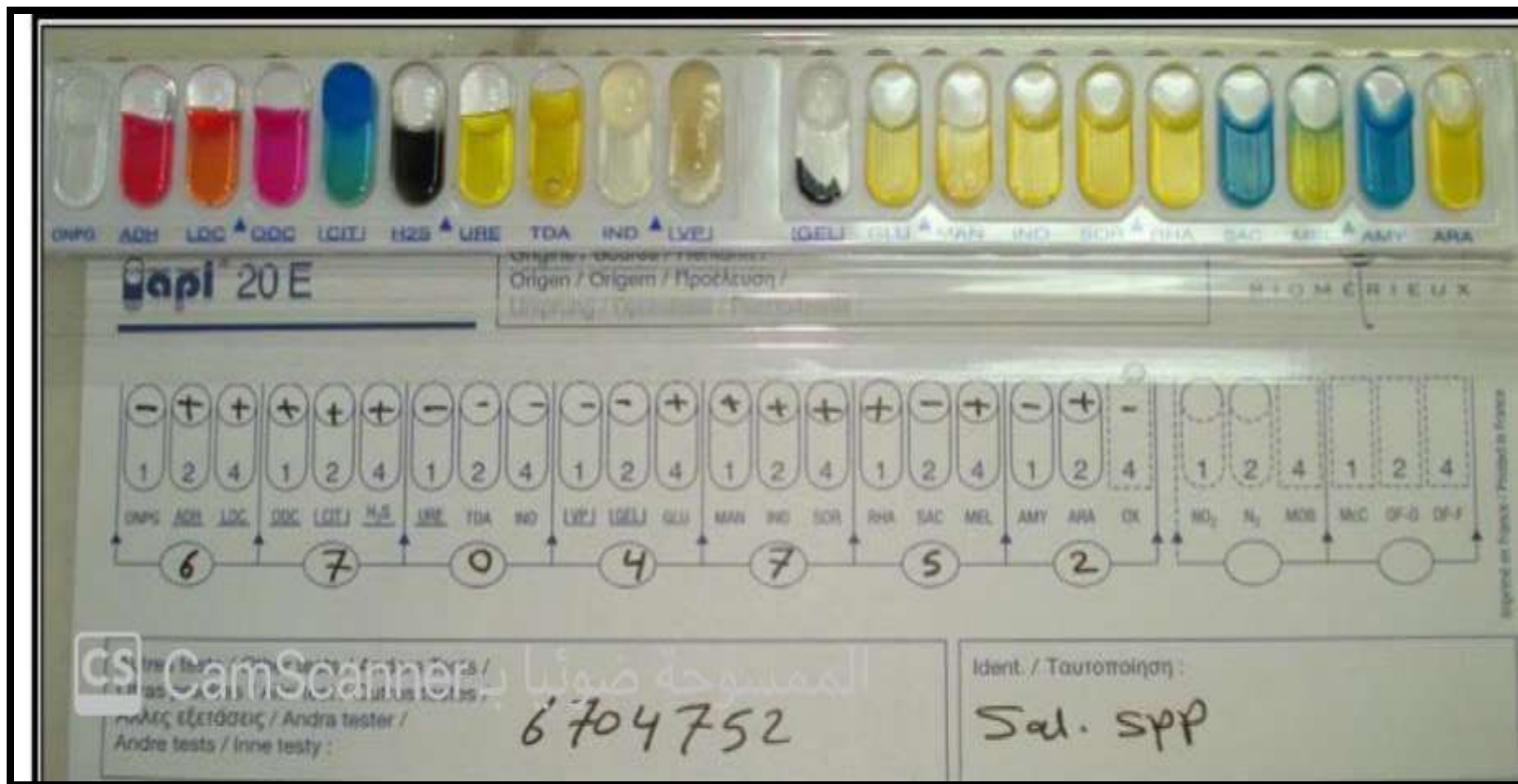


Figure (3) : API 20E result

The API system

Example of results for bacteria to test :

1-Reading results :



2-Entering results in the database software:

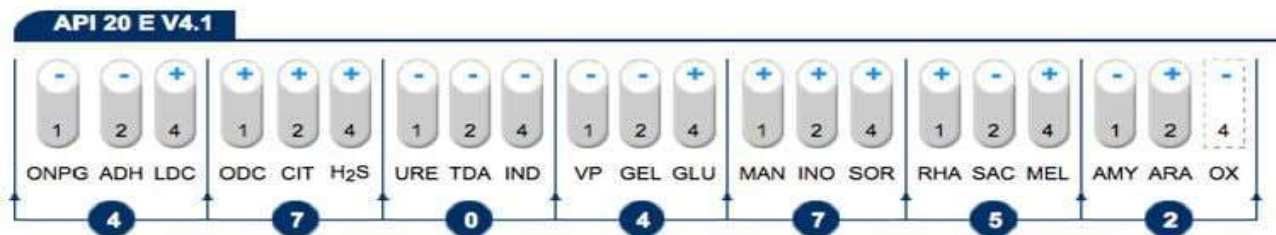


Figure (4) : API 20E result

Thanks
for
Listening

كلية النور الجامعة

قسم تقنيات المختبرات الطبية

Diagnostic Microbiology

المرحلة الرابعة

Lab 13 /2023



Antimicrobial Susceptibility Test

Diagnostic Microbiology

Lab: 13

Assist lecture: Nadia Khalid Mustafa

Antimicrobial susceptibility testing:

is a standardized method that is used to measure the effectiveness of antibiotics and other chemotherapeutic agents on pathogenic microorganisms.

it is an essential tool in describe appropriate treatment.

Types of drugs according to the nature of their composition and origin:

- 1- Antibiotics:** Produced by some microbes naturally as a by-product of their metabolic activities, and have the ability to inhibit or kill other types of microbes, like: Penicillin and Cephalosporin.
- 2- Synthetic agents:** It includes agents that are completely synthesized in laboratories by chemical processes unrelated to microbial activity, like: Ciprofloxacin.
- 3- Semi synthetic agents:** They are natural antibiotics that have been chemically modified, like: Ampicillin.

According to the mechanisms of action of the drugs, drugs can be categorized into several general groups:

- 1- Drugs that inhibit **cell wall** synthesis, like: Penicillin.
- 2- Drugs that inhibit **cytoplasmic membrane**, like: Colistin.
- 3- Drugs that inhibit **protein** synthesis, like: Tetracycline.
- 4- Drugs that inhibit **nucleic acid** synthesis, like: Rifamycin.
- 5- Drugs that inhibit **folic acid** synthesis, like: Sulfonamides.

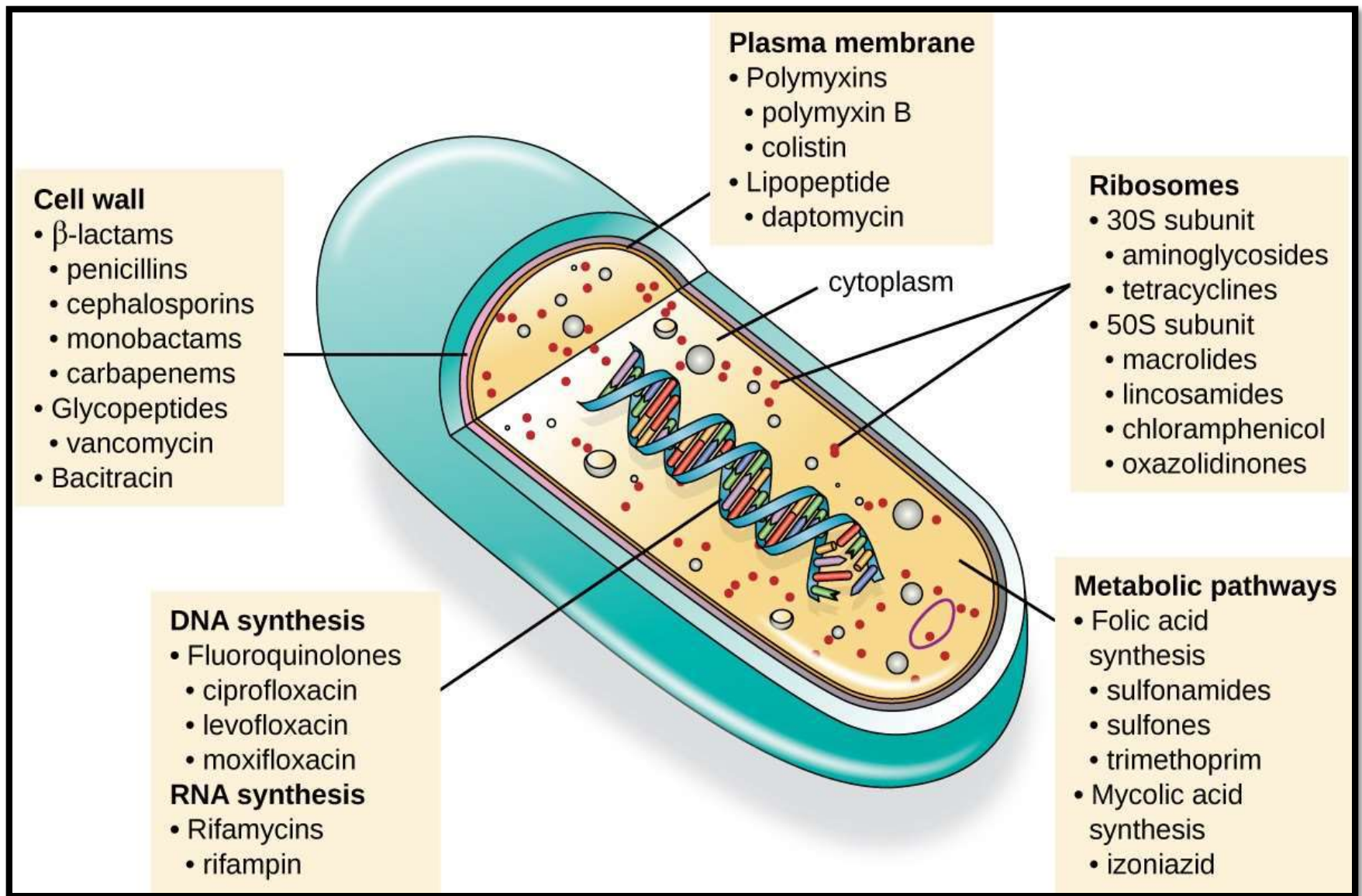


Figure (1) : The mechanisms of action of the drugs

According to **spectrum of action**, drugs classified into:

1- narrow spectrum drugs: drugs that work against only a few kinds of pathogens, like: penicillin, it affects only gram positive bacteria.

2- Broad spectrum drugs: drugs that effect against many different kinds of pathogens, like: Ampicillin.

According to **mode of action**, drugs can be classified into:

1- Bacteriostatic: drugs that stop or inhibition the growth, but don't kill the microbe.

2- Bactericidal: drugs that kill the organism.

The disk diffusion test (The Kirby-Bauer test):

Is a valuable standard tool for measuring the effectiveness of antimicrobials against pathogenic microorganisms.

Procedure:

- 1-** Select a pure culture plate of one of the organisms to be tested.
- 2-** Transfer a colony from the plate to the sterile saline solution, mix it well.
- 3-** To titrate the inoculum density, a standard turbidity tube (McFarland Tube No. 0.5) is used.

McFarland Standards

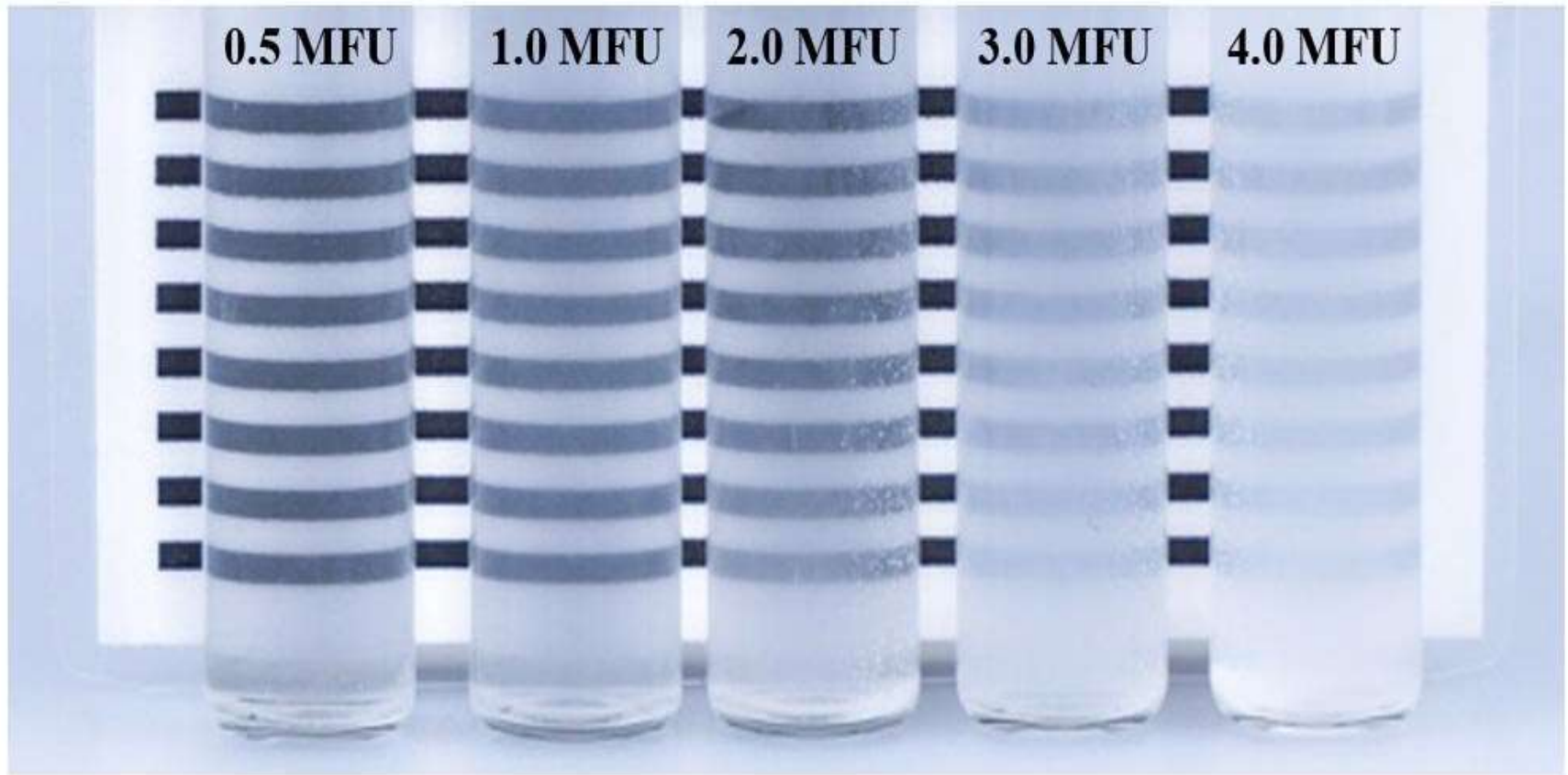


Figure (2) : McFarland standards

- 4-** Take a sterile swab and dip it into the broth culture of organism.
- 5-** Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab.
- 6-** Use the swab with the test organism to streak a Mueller-Hinton agar (MHA) plate or a NA plate.
- 7-** After the streaking is complete, allow the plate to dry for 5 minutes.
- 8-** Antibiotic discs can be placed on the surface of the agar using sterilized forceps and press the discs onto the surface of the agar.
- 9-** Invert the inoculated plates and incubate for 24 hours at 37°C.
- 10-** After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.

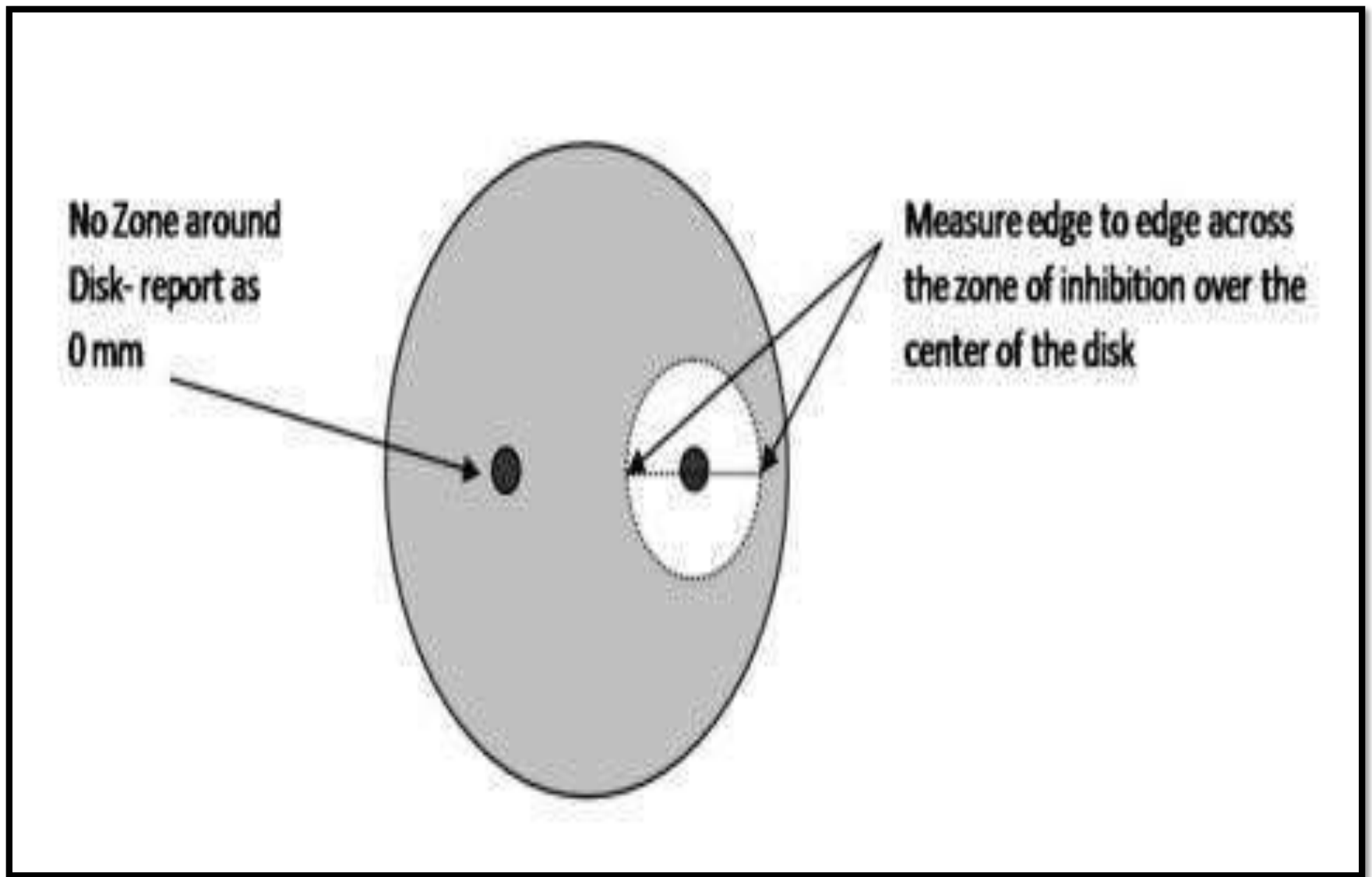


Figure (3) : the zone of inhibition

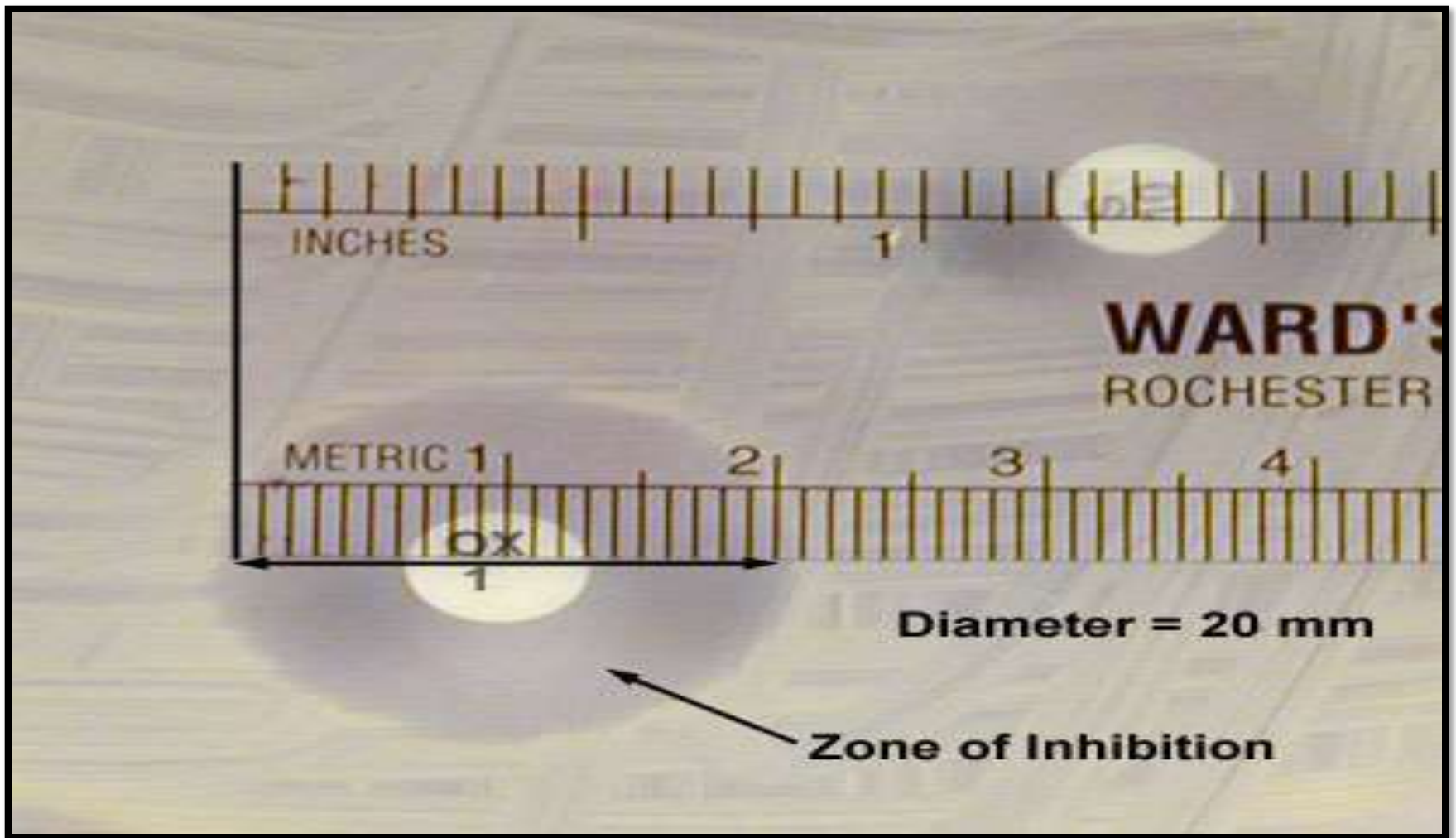


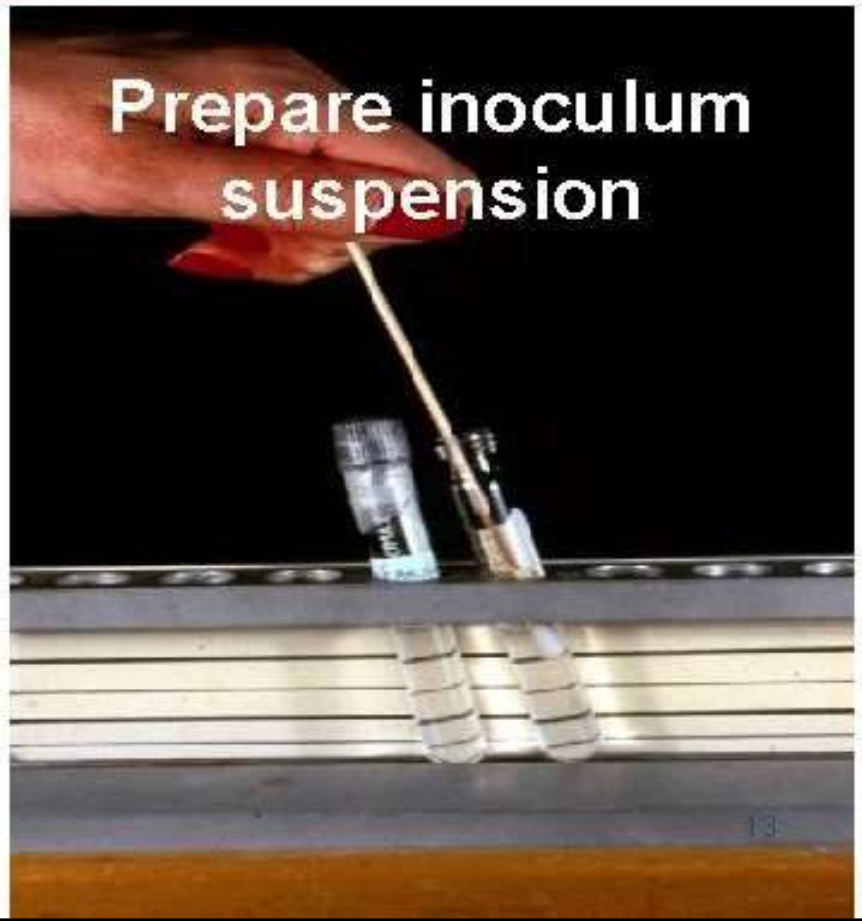
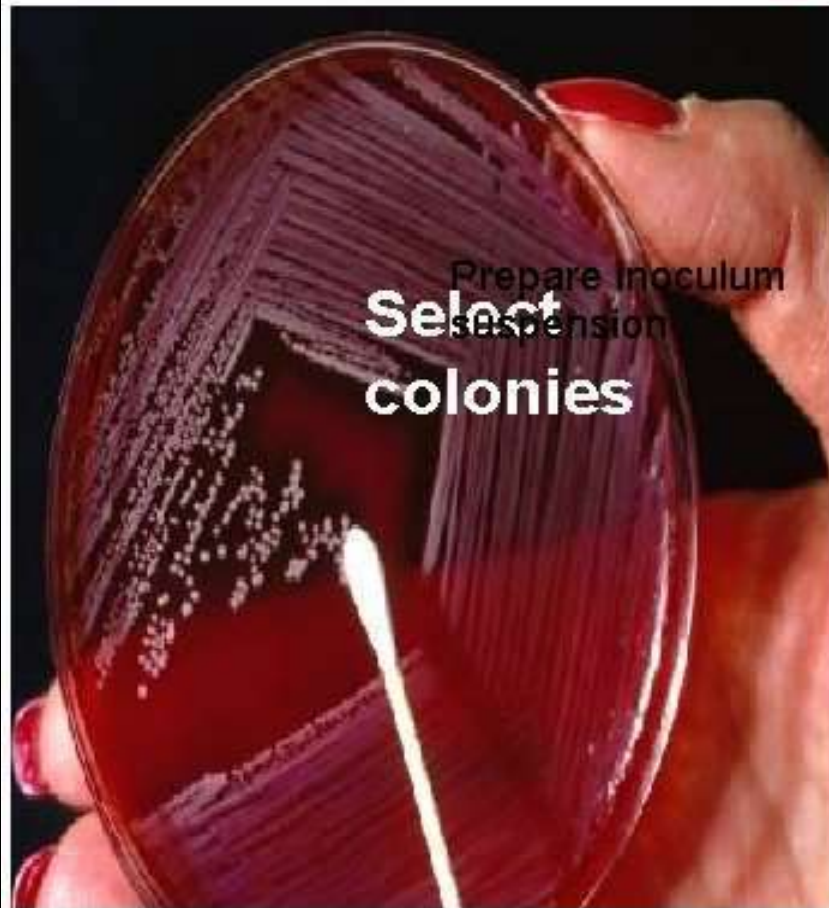
Figure (4) : measure the diameter of the zone of inhibition

11- Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone and whether the tested bacterial species is Sensitive(S), intermediate (I) or Resistant(R) to the tested antibiotic.

Antimicrobial agent	Symbol	Disc content	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Amikacin	Ak	30 mcg	14	15-16	17
Ampicillin	As	10 mcg	11	12-14	15
Chloramphenicol	C	30 mcg	12	13-17	18
Gentamicin	G	10 mcg	12	13-14	15
Kanamycin	K	30 mcg	13	14-17	18
Lincomycin	L	2 mcg	9	10-14	15
Nalidixic acid	Na	30 mcg	13	14-18	19
Novobiocin	Nv	30 mcg	17	18-21	22
Penicillin G	P	1 mcg	14	15-18	19
Streptomycin	S	10 mcg	11	12-14	15
Tetracycline	T	30 mcg	14	15-18	19
Vancomycin	Va	30 mcg	14	15-16	17

Figure (5) : Zone size interpretation chart for (Kirby-Bauer) test

Disk Diffusion Test



Prepare the Material for Inoculation

Standardize inoculum
Suspension as per Mac farland
standard

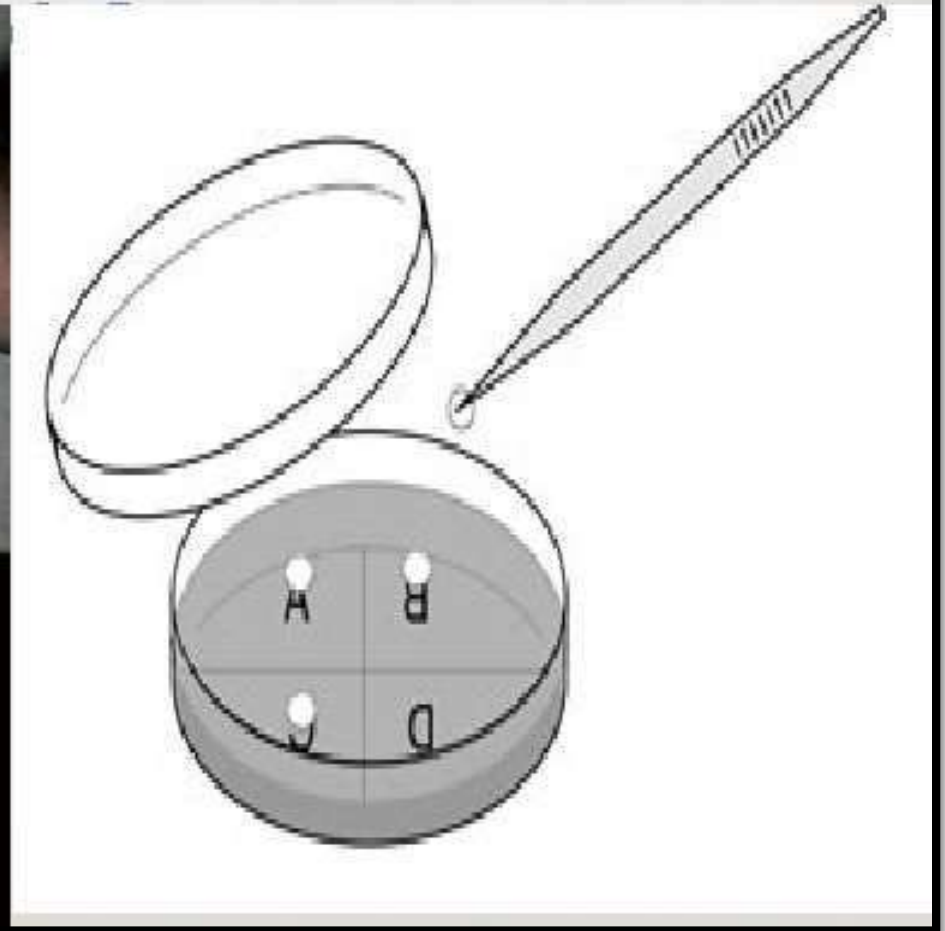
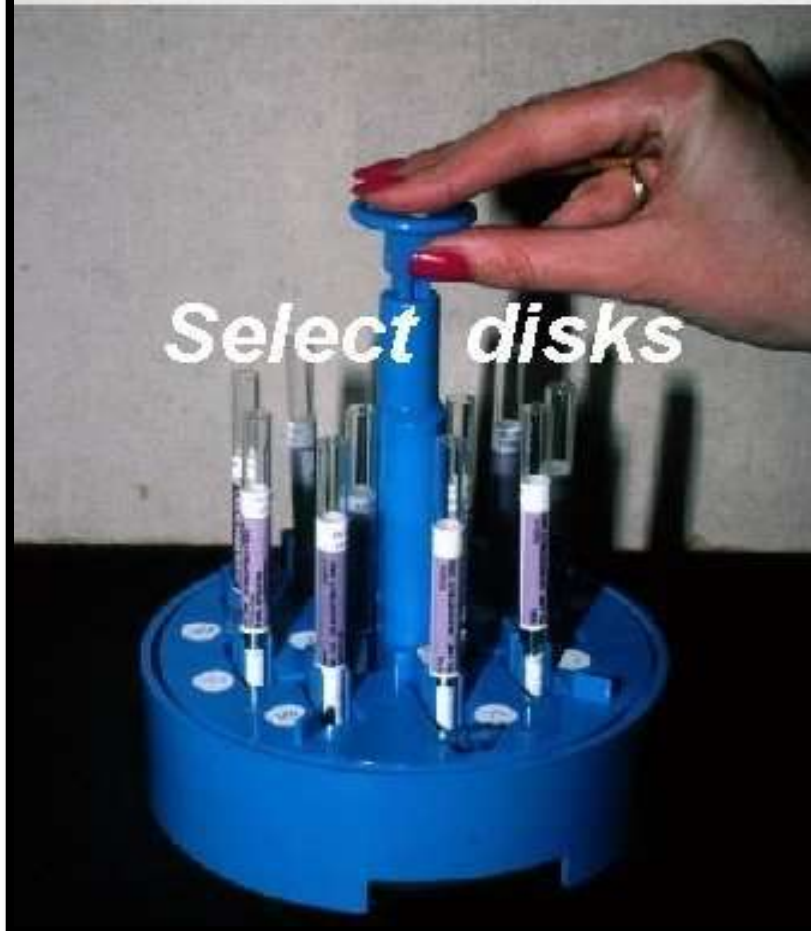
Mix well



Swab the plate with optimal sample

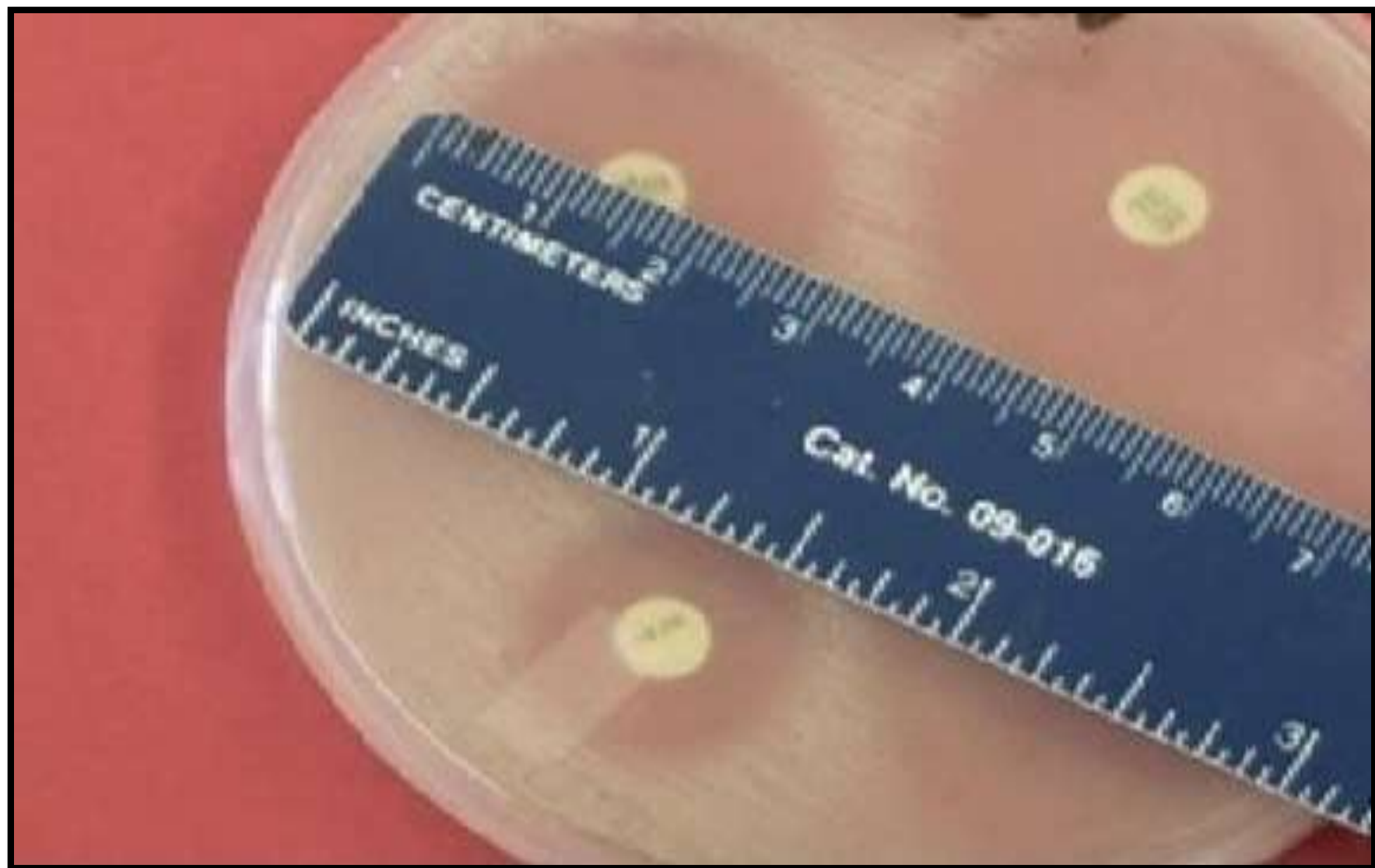


Select the Disks and Apply



Incubate Overnight





Thanks
for
Listening

Practical Diagnostic Microbiology

Lab. 14

The fourth stage.

Laboratories Techniques.

Bacteremia and Septicemia

Bloodstream infections (BSIs), which include bacteremia and other infection like fungi...etc. are present in the blood.

Blood is normally a sterile environment, so the detection of microbes in the blood is always abnormal.

Bacteremia: is the presence of bacteria in the bloodstream that are alive and capable of reproducing.

Septicemia: is a serious bloodstream infection. It's also known as **blood poisoning**, the bacteria and their toxins can be carried through the bloodstream to your entire body.

Bacteria can enter the bloodstream by:

- severe complication of infections (like: pneumonia or meningitis).
- during surgery.
- due to catheters and other foreign bodies entering the arteries or veins including during intravenous drug.
- after dental procedures or brushing of teeth.

The most important types of microorganisms that infect the blood:

- **gram-positive bacteria:** Staphylococcus, Streptococcus, and Enterococcus.
- **gram-Negative bacteria:** *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis*.

- **Fungi:** *Candida albicans*.

Diagnosis bacteremia:

- disinfected the area using an **alcohol** to prevent contamination and left to dry followed by **iodine** to avoid contamination.
- A typical blood culture collection involves drawing blood into **two bottles**, One bottle is designed to growth of aerobic organisms, and the other is designed to grow anaerobic organisms.
- incubated for up to **five days**.
- **Culture** on the media agar.

Note: because antimicrobial therapy can cause **false negative** results by inhibiting the growth of microbes, it is recommended that blood cultures are drawn before antimicrobial drugs are given.

Good Luck

Prepared by Asst. Lec. Dunya W. Almshlh

كلية النور الجامعة

قسم تقنيات المختبرات الطبية

Diagnostic Microbiology

المرحلة الرابعة

Lab 15 /2023



Neisseria sp.

Diagnostic Microbiology

Lab: 15

Assist lecture: Nadia Khalid Mustafa

This genus was first discovered by the German scientist **Albert neisser** in 1879, the two most important species were:

Neisseria gonorrhoeae and

Neisseria meningitides.

Collect the sample:

Blood,

Urethral swab

and C.S.F.

Isolation:

Neisseria meningitides: Blood agar and Chocolate agar.

Neisseria gonorrhoeae: Thayer marten media (TM), New York city media (NYC), Martin Lewis media (ML).

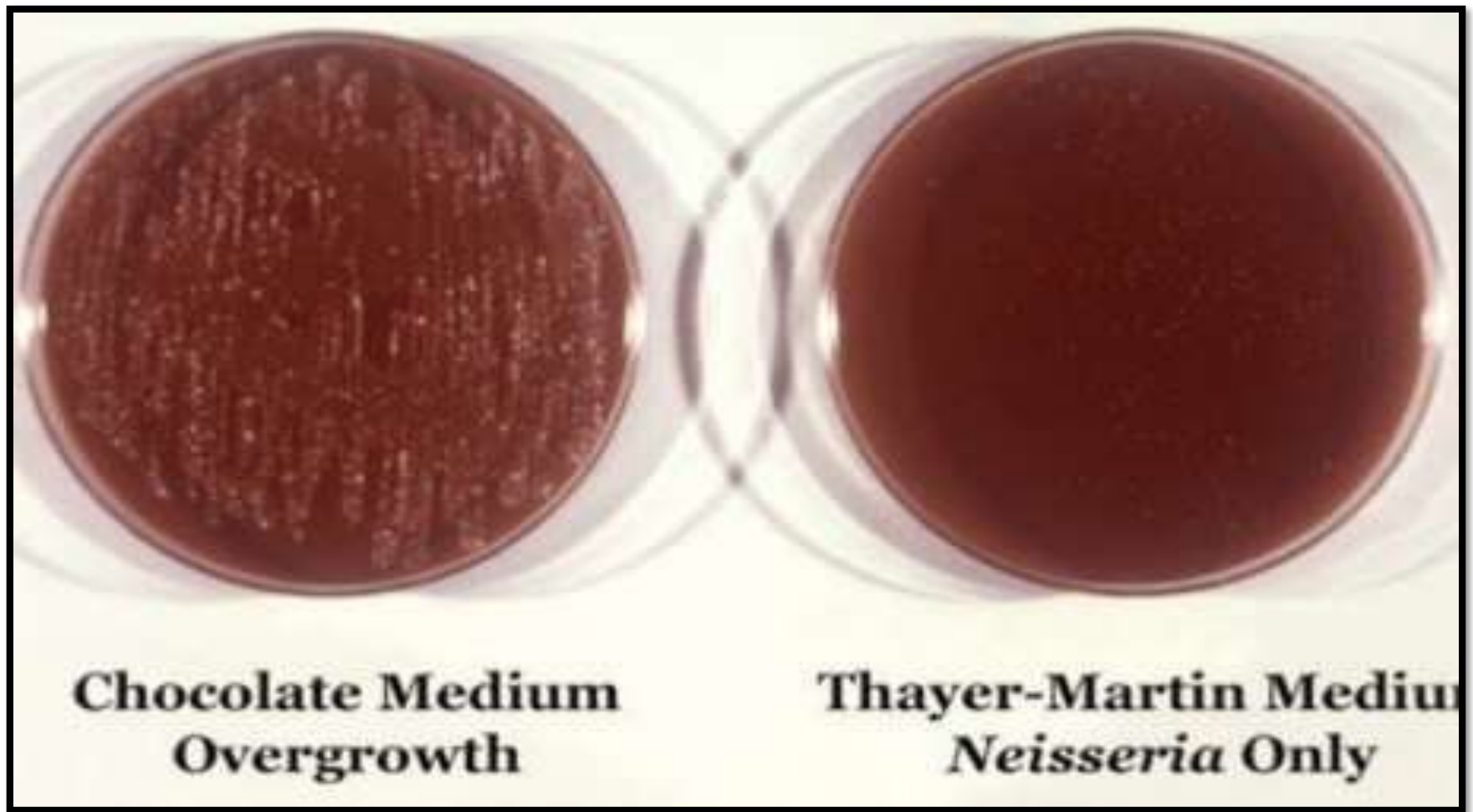


Figure (1) : *Neisseria gonorrhoeae* on TM media

Culture characteristics:

General characteristics: Aerobic, some of its strains are CO₂ loving **Capnophilic**, oxidase positive.

Neisseria meningitidis: also called **Meningococcus** its colonies appear as mucous in a **grayish or yellowish** color, **capsulated**, the acid is produced from the fermentation of **glucose and maltose**, cause **meningitis**.

Neisseria gonorrhoeae: also called **Gonococcus** its colonies **colorless, non- capsulated**, It produces acid from **glucose** fermentation only, cause **gonorrhea**.

Microscopy characteristics:

Spherical or oval Gram negative, non-motile, its cells are arranged in flat-sided contiguous pairs **Diplococci** , or the cells may be in pairs with frustration on both sides in a shape similar to a **bean or a kidney shaped**.

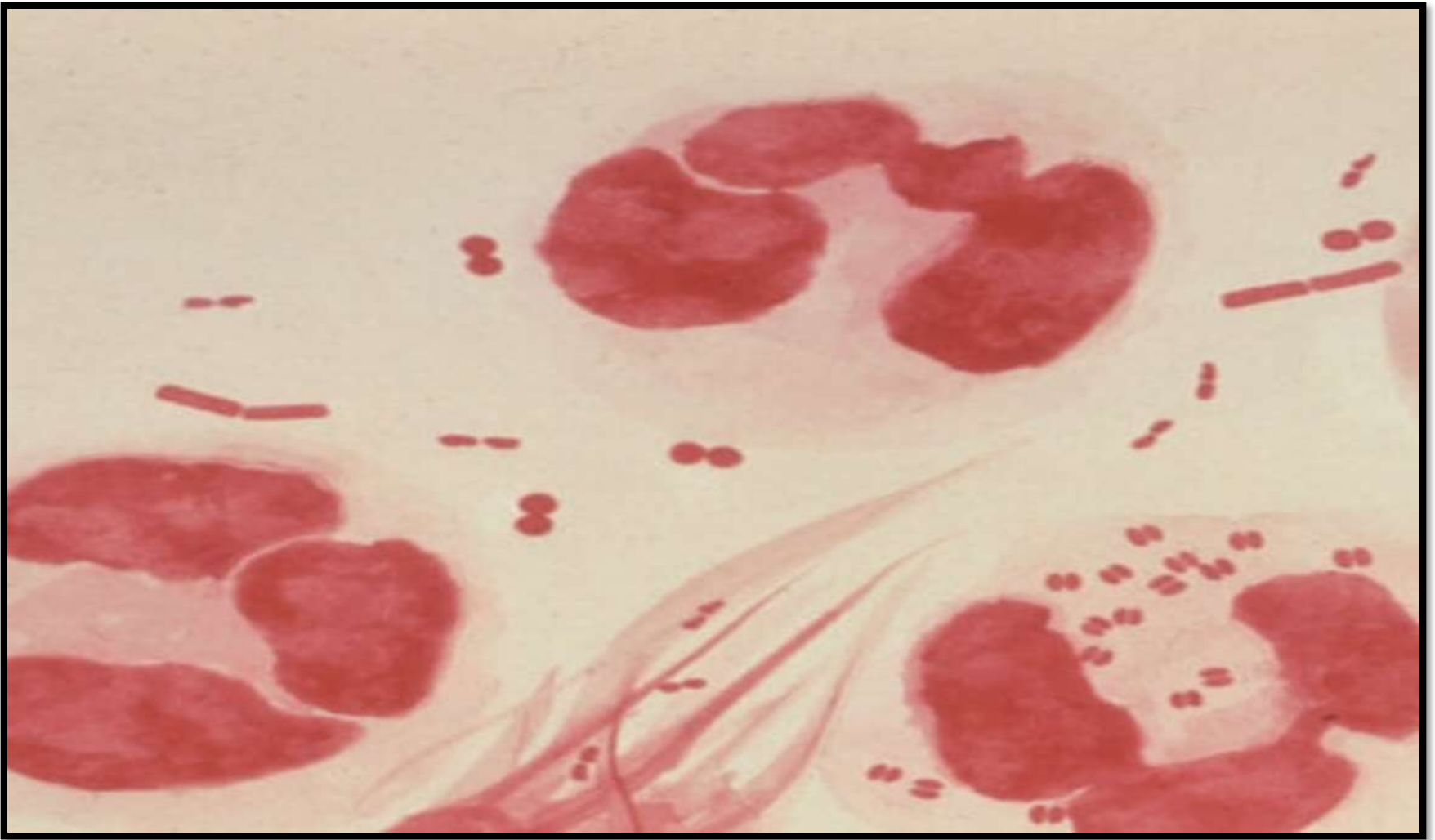


Figure (2) : Neisseria sp. under the microscope

Thanks
for
Listening