وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الجنوبية
المعهد التقني العمارة
قسم تقنيات المختبرات الطبية

الحقيبة التدريسية لمادة علم الأحياء المجهرية العملي

الصف: الثاني

تدريسي المادة

م.د. رواء صادق مجيد

الفصل الدراسي الأول

جدول مفردات مادة علم الأحياء المجهرية العملي

Practical syllabus	
weeks	Topics
1	Introduction, behavior inside Lab.
2	Sterilization and disinfection methods.
3	Specimen Collection and Processing
4	Microscopic Examination of Infected Materials
5	Use of Colonial Morphology for the Presumptive Identification of Microorganisms.
6	Biochemical Identification of Bacteria
7	Immunological methods used for microorganism detection
8	Applications of Molecular Diagnostics, NUCLEIC ACID HYBRIDIZATION TECHNIQUES
9	NUCLEIC ACID AMPLIFICATION PROCEDURES
10	Other Nucleic Acid Amplification Reactions, Nucleic Acid Sequence Based Amplification
11	Antimicrobial Susceptibility Testing, SELECTING ANTIMICROBIAL AGENTS FOR TESTING, Reporting of Susceptibility Test Results.
12	TRADITIONAL ANTIMICROBIAL SUSCEPTIBILITY TEST METHODS, Inoculum Preparation and Use of McFarland Standards, Dilution Susceptibility Testing Methods, Antimicrobial Stock Solutions, Broth- Macrodilution (Tube-Dilution) Tests, Agar-Dilution Tests
13	Disk Diffusion Testing, Principle, Establishing Zone- Diameter. Interpretive Breakpoints, Disk Storage, Inoculation and Incubation, Reading Plates and Test Interpretation
14	Modified Methods for Testing Slow-Growing or Fastidious Bacteria
15	Susceptibility Testing of Anaerobes

الهدف من دراسة مادة علم الأحياء المجهرية (الهدف العام):

تهدف دراسة مادة علم الأحياء المجهرية العملي للصف الثاني إلى:

التعرف على الأحياء المجهرية التي تسبب الأمراض للإنسان وأمراضها وتشخيصها.

الفئة المستهدفة: طلبة الصف الثاني/قسم تقنيات المختبرات الطبية.

التقنيات التربوية المستخدمة:

1- سبورة وأقلام.

2- السبورة التفاعلية.

3- عارض شاشة Data Show.

4- جهاز حاسوب محمول Laptop.

الأسبوع الأول

الهدف التعليمي: التعرف على الأحياء المجهرية الطبية المسببة الأمراض للإنسان وتشخيصها.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة **Introduction to Medical Microbiology**

Medical microbiology is a branch of microbiology that focuses on the study of microorganisms and their interactions with the human body, particularly in the context of health and disease. It plays a crucial role in understanding, diagnosing, and treating infectious diseases.

Microorganisms, also known as microbes, are tiny, living organisms that cannot be seen with the naked eye. They encompass a diverse group of life forms, including bacteria, viruses, fungi, and parasites. While many microbes are harmless and even beneficial to humans, some can cause diseases, making them the primary focus of medical microbiology.

Behavior Inside Microbiology Laboratory:

1. Wash your hands with disinfectant soap when you arrive to the lab and again before you leave.
2. Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory. Do not put anything in your mouth such as pencils, pens, labels, or fingers. Do not store food in areas where microorganisms are stored.
3. Wear a lab coat and safety glasses, bring them to lab and use them.
4. Wear appropriate shoes (sandals are not allowed) in the laboratory.
5. Keep your workspace free from all unnecessary materials.

6. Disinfect work area before and after use with 70% ethanol.

7. Label everything clearly.

8. Do not open Petri dishes in the lab unless absolutely necessary.

9. Inoculating loops and needles should be flame sterilized in a Bunsen burner before you lay them down.

10. Turn off Bunsen burner when is not use.

11. When you flame sterilize with alcohol, be sure that you do not have any papers under you.

12. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.

13. Wear disposable gloves and mask when working with potentially infectious microbes or samples.

14. Sterilize equipment and materials.

15. Never pipette by mouth.

16. Consider everything a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding.

17. Dispose of all solid waste material in a biohazard bag and autoclave it before discarding in the regular trash.

18. Dispose of broken glass in the broken glass container.

19. Dispose of syringe needles and sharp metal objects in the “sharps” container.

20. Report spills and accidents immediately to your instructor. Clean small spills with care . Search help for large spills.

21. Report all injuries or accidents immediately to the instructor.

الأسبوع الثاني

الهدف التعليمي: التعرف على طرق التطهير والتعقيم.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Sterilization and Disinfection Methods:**

Sterilization: removal or killing of all living microorganisms including bacteria and their spores.

Disinfection: removal or killing of disease-causing microorganisms (not necessarily all microorganisms).

Bactericidal: kills bacteria.

Bacteriostatic: inhibits growth of bacteria.

Methods of Sterilization:

There are two methods of sterilization:

1- Physical Methods of Sterilization:

a- Sterilization by Heat:

- Heat is the most practical, reliable, and inexpensive method of sterilization.

- It is used for sterilization of objects and materials that can tolerate high temperatures.

- It can be either dry heat (red heat, flaming, incineration and hot air oven) or moist heat (pasteurization, boiling and autoclaving).

Dry Heat:

- Red Heat:

Principal:

Holding the object in the flame of Bunsen burner till they become red hot.

Used for:

Sterilization of bacteriological loops and tips of forceps.

- Flaming:

Principal:

Passing the object through the flame of Bunsen burner without heating to redness.

Used for:

Sterilization of glass slides and mouth of culture tubes.

- Incineration:

Principal:

Infectious materials are converted to sterile ash by burning in incinerator.

Used for:

Destruction of contaminated disposable materials (waste).

- Hot Air Oven:

Principal

- Objects to be sterilized are exposed to high temperature in an electrically heated oven.

- Even distribution of heat throughout the chamber is achieved by a fan.

Holding Time:

160 °C for two hour.

180 °C for one hour.

Used for:

- Sterilization of all glasses: test tubes, Petri dishes, flasks and pipettes.

- Sterilization of instruments: forceps, scalpels and scissors.

- Sterilization of materials in closed containers: fat, oils and powder.

Moist Heat:

- Pasteurization: (Below 100 °C).

Principal:

It is a process of heating a liquid to a specific temperature for a definite length of time and then cooling it immediately.

Used for:

It is commonly used in milk processing.

- Boiling: (At 100 °C).

Principal:

Boiling in water for fifteen minutes.

Used for:

Kill most vegetative bacteria and inactivate viruses.

* However boiling is ineffective against many bacterial and fungal spores.

- Autoclaving:

Principal:

- When the pressure is increased inside a closed container, the temperature at which water boils exceeds 100 °C.

- At double atmospheric pressure, the temperature of the steam reaches 121°C.

- Autoclaving is the most reliable method of sterilization that kills all kinds of bacteria and spores.

Temperature of Sterilization:

121°C for 15 minutes and 15 psi**.**

Used for:

- Sterilization of culture media.

- Sterilization of surgical supply: e.g. dressing and surgical instruments.

b- Sterilization by Filtration:

Principal:

It is possible to remove bacteria from fluids by passing them through filters with pores so small that bacteria are arrested.

Used for:

It is used to sterilize liquids that would be damaged by heat as sera, antibiotic solutions and vaccines.

c- Sterilization by Irradiation:

- Non- Ionizing irradiation: e.g. ultra violet (UV) ray.

- Ionizing irradiation: e.g. gamma ray.

Non- Ionizing Irradiation (Ultra violet (UV) ray):

Used for:

- Sterilization of operating theatre.

- Sterilization of the interiors of biological safety cabinets.

Ionizing Irradiation (Gamma ray):

Used for:

Sterilization of an object not tolerate heat as rubber catheters, gloves and plastic syringes.

2- Chemical Methods of Sterilization:

Disinfectants: are chemical materials used for sterilization but are toxic to the human tissues and cells.

Antiseptics: are chemical materials used for sterilization but not toxic to the human body e.g. "mouth washes".

Examples of Disinfectants and Antiseptics:

There are a number of chemicals that can act as disinfectants or antiseptics. These include:

- Phenol and its derivatives: e.g. Dettol.

- Halogens: e.g. chlorine and tincture of iodine.

- Alcohols: e.g. ethyl alcohol.

- Aldehydes: e.g. glutaraldehyde (Cidex) and formalin.

- Quaternary ammonium compounds: e.g. cationic detergents.

الأسبوع الثالث

الهدف التعليمي: التعرف على كيفية جمع العينات ومعاملتها.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Specimen Collection and Processing**

1- Blood Collection:

There are three general procedures for obtaining of blood:

a- Capillary Blood Collection: is frequently used when only small quantities of blood are required, e.g., for hemoglobin quantitation, for WBC and RBC counts and for blood smear preparation. It is also used when venipuncture is impractical, e.g. in infants, in cases of severe burns, in extreme obesity where locating the veins could be a problem and in patients whose arm veins are being used for intravenous medication.

Sites of Puncture:

1- Adults and children: palmar surface of the tip of the ring or middle finger or free margin of the ear lobe.

2- Infants: plantar surface of the big toe or the heel.

b- Venous Blood Collection: is used for most tests that require anticoagulation or larger quantities of blood, hematology plasma or serum.

Sites of Puncture:

1- The veins that are generally used for venipuncture are those in the forearm, wrist or ankle.

2- In infants and children, venipuncture presents special problems because of the small size of the veins and difficulty controlling the patient. Puncture of the external jugular vein in the neck region and the femoral is the procedure of choice for obtaining blood.

c- Arterial Blood Collection: is used to measure oxygen and carbon dioxide tension, and to measure pH . These blood gas measurements are critical in assessment of oxygenation problems encountered in patients with pneumonia, pneumonitis, and pulmonary embolism.

* Arterial puncture is technically more difficult to perform than venous puncture. Increased pressure in the arteries makes it more difficulty to stop bleeding with the undesired development of a hematoma.

Anticoagulants: are chemical substances that are added to blood to prevent coagulation.

Types of Anticoagulants:

1- Ethylene diamine tetra acetic acid (EDTA): used to platelet counts and platelet function tests since it prevents platelet aggregation.

2- Tri sodium citrate (TSC): used to the erythrocyte sedimentation rate (ESR).

3- Heparin: used to osmotic fragility test and hematocrit determination .

*** Different between Plasma and Serum:**

Plasma: the blood with anticoagulant.

Serum: the blood without anticoagulant.

2- Urine Collection:

- Containers for the collection of urine should be wide-mouthed, clean and dry.

- The first voided morning specimen is preferred because it is usually more concentrated and therefore more likely to reveal abnormalities.

- In the midstream urine collection method while patient urinates places an open in the stream of urine and collects about 20 ml of urine. The container should be covered immediately.

3- Stool Collection:

- A clean dry container must be used for the collection of stool specimens.

- The specimen should be brought to the lab as soon as it is passed.

- Diarrhea specimens or those containing blood and mucous should be examined promptly on arrival to the lab.

Sample Processing:

Sample processing encompasses the steps taken to prepare and handle samples, from collection to analysis, ensuring they are suitable for testing and yield reliable results. This involves various procedures like labeling, preserving, transporting, and potentially concentrating or cleaning up the sample. In essence, it transforms a sample into a usable form for specific analytical methods.

1- Sample collection and labeling: proper collection techniques , including using appropriate tools and adhering to volume requirements , are crucial . Accurate and complete labeling with unique IDs, date, time, and other relevant details is essential to prevent errors.

2- Sample preservation and storage: depending on the type of sample and analysis , preservatives may be needed, and proper storage conditions (temperature, etc.) must be maintained to prevent degradation.

3- Transportation: samples need to be transported securely and in a manner that maintains their integrity. This may involve using specialized containers and following specific protocols.

4- Sample preparation: this stage involves transforming the sample into a form that can be directly analyzed by the chosen method. It may include steps like extraction, filtration,

homogenization, or concentration.

5- Quality control: throughout the process, quality control measures are implemented to verify sample integrity and ensure the reliability of the results.

الأسبوع الرابع

الهدف التعليمي: التعرف على الفحص المجهري للمواد المصابة.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Microscopic Examination of Infected Materials**

1- Blood:

Laboratory blood analysis is one of the most important routine diagnostic procedures in a clinical lab. Hematologists routinely examine peripheral blood smears on glass slides with a microscope to find abnormalities in morphological characteristics of cells and tissues indicating hematological diseases or to look for blood parasites, such as those found for malaria and filariasis. A microscopic image can deliver information on cell types based on their morphology, about the quantity and composition of blood cells. Light microscopy with a magnification of up to 1,000X is employed to recognize and count the various cell types within a monolayer and document the results with a digital camera. This way, many types of blood diseases or the developmental stages of parasites can be visualized.

Blood Smear Preparation:

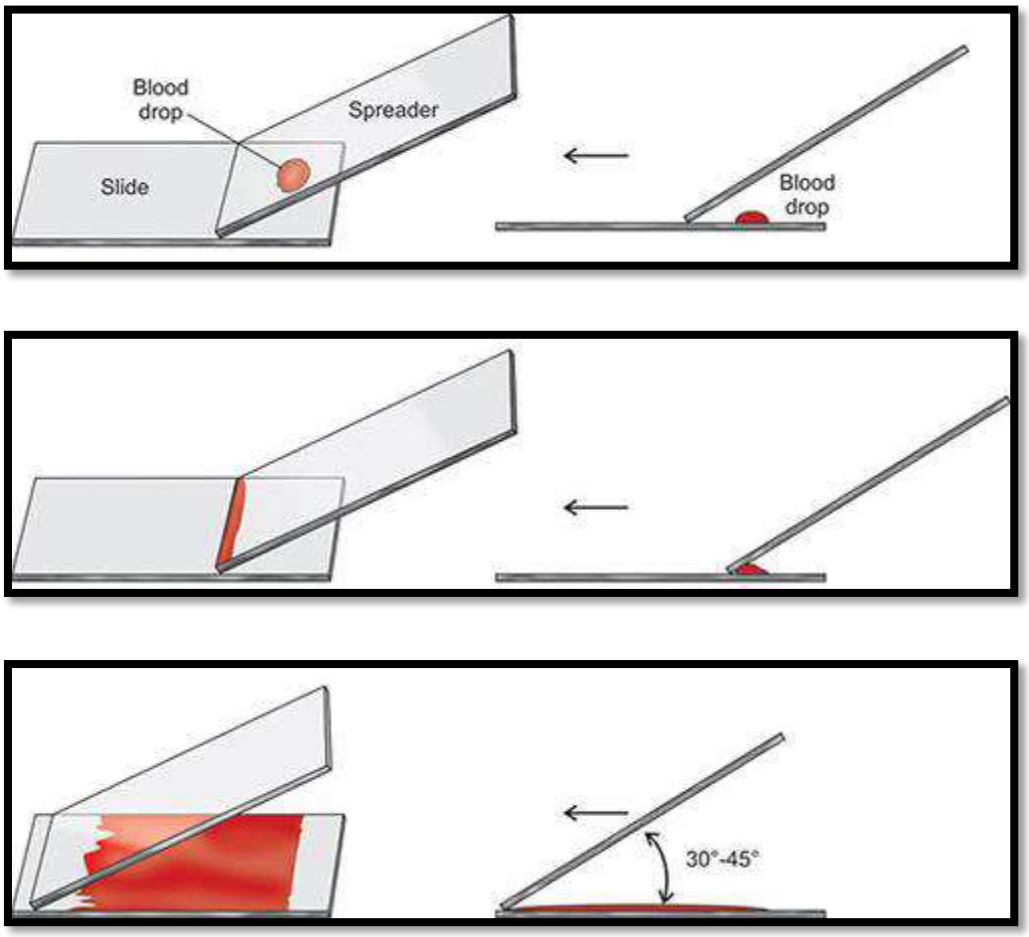
A blood smear is used to look for abnormalities in blood cells, be it their morphology or quantity. It can help to detect, diagnose, and monitor deficiencies, diseases, and disorders involving blood cell production, function, and lifespan. Normally a microscopic analysis of a thin blood smear is done when the complete blood count or the differential white blood count deliver abnormal results.

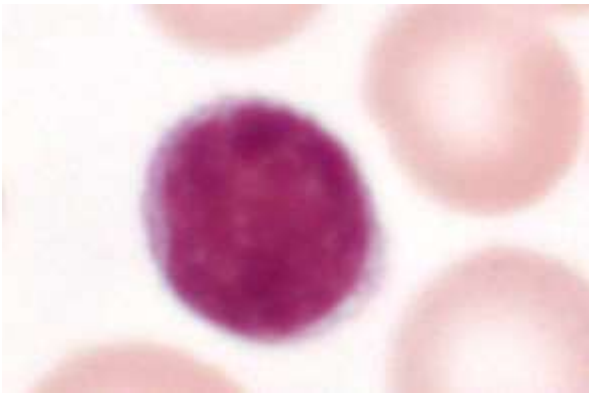
1- Place a clean glass slide on a flat surface. Add a small drop of blood from, for example, the fingertip.

2- Place the cover slip in an angle of about 30 - 45°.

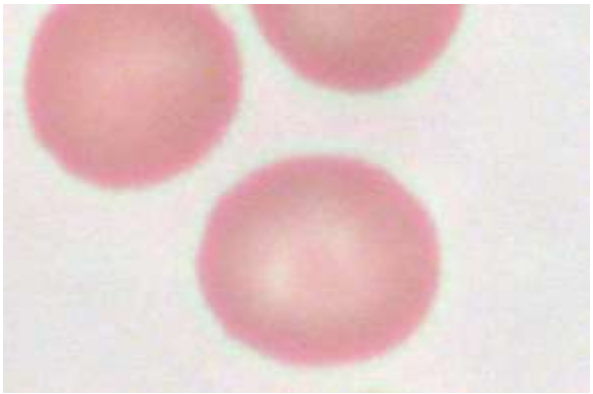
3- Gently spread the edge of the cover slip over the blood and produce a smear.

4- The smear is air-dried, fixed to the slide with methanol, and stained with Wright's stain, Leishman stain, or Giemsa stain to distinguish the various cell types.

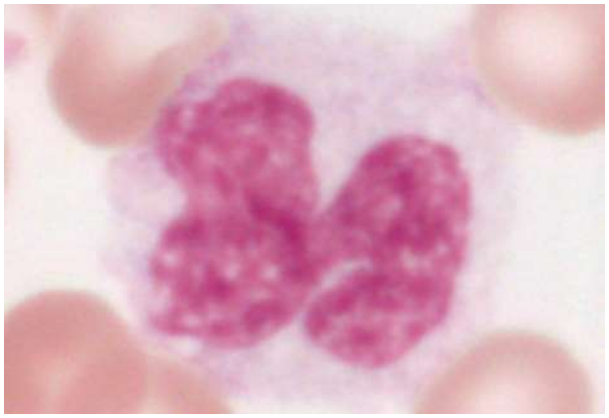




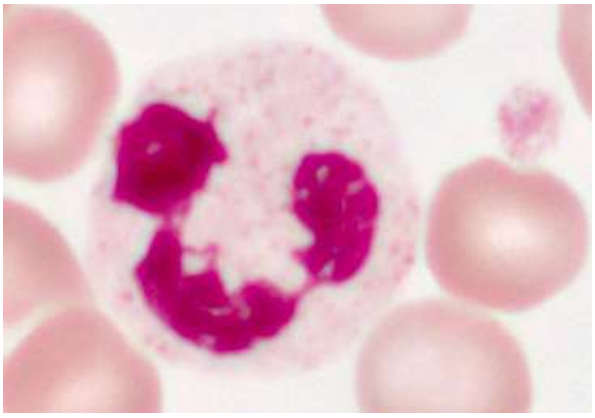
Lymphocyte



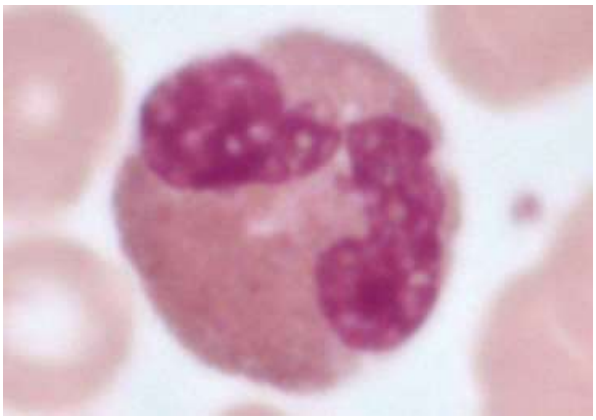
Erythrocyte



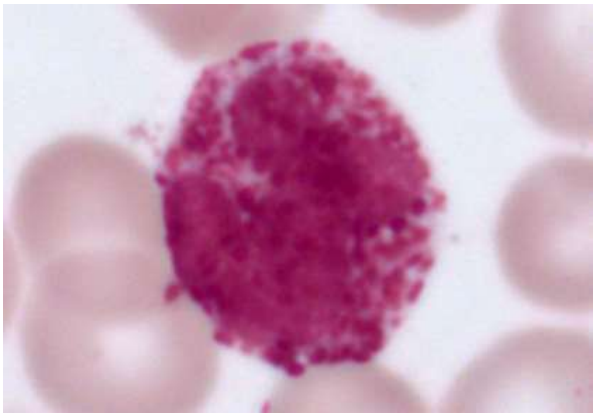
Monocyte



Neutrophils



Eosinophils



Basophils

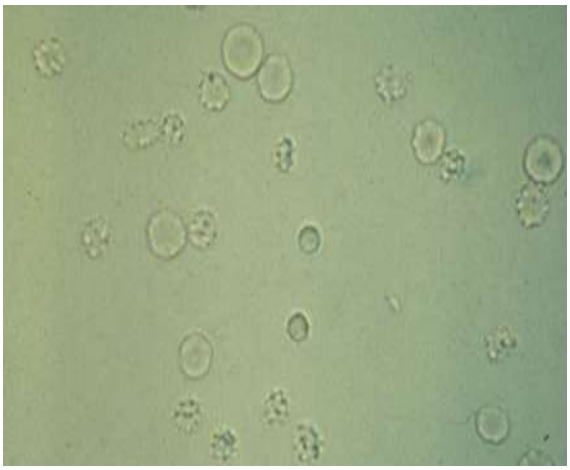
2- Urine:

1- A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed about 2000-3000 rpm for 5-10 minutes which produces a concentration of sediment (cellular matter) at the bottom of the tube.

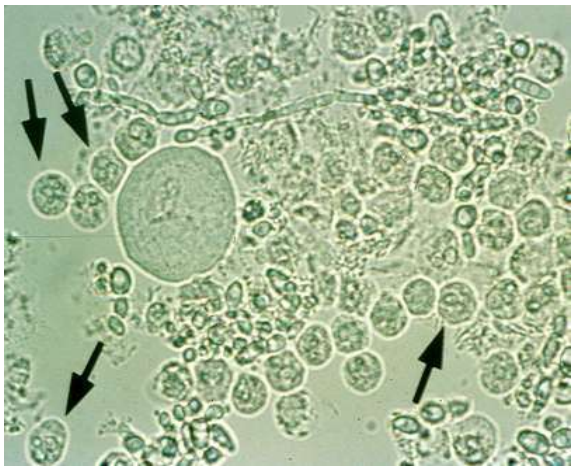
2- A drop of sediment is poured onto a glass slide, a thin slice of glass (a cover slip) is place over it and observed under microscope.

3- A variety of normal and abnormal cellular elements may be seen in urine sediment such as:

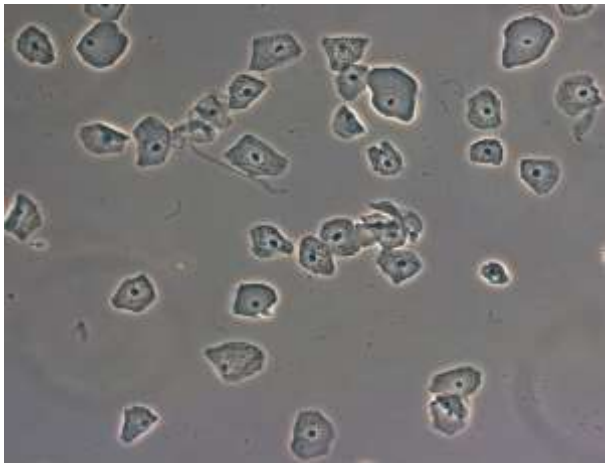
- Red blood cells.
- White blood cells.
- Mucus.
- Various epithelial cells.
- Various crystals.
- Bacteria.
- Casts.



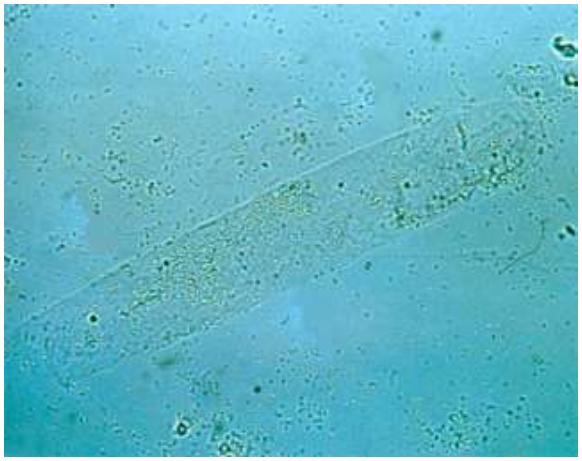
Red blood cells appear as refractile disks



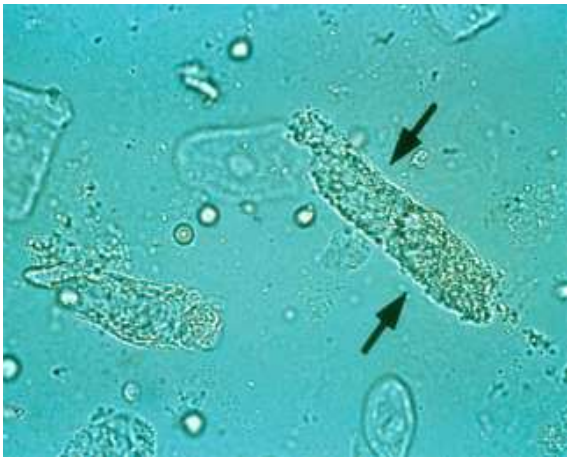
White blood cells



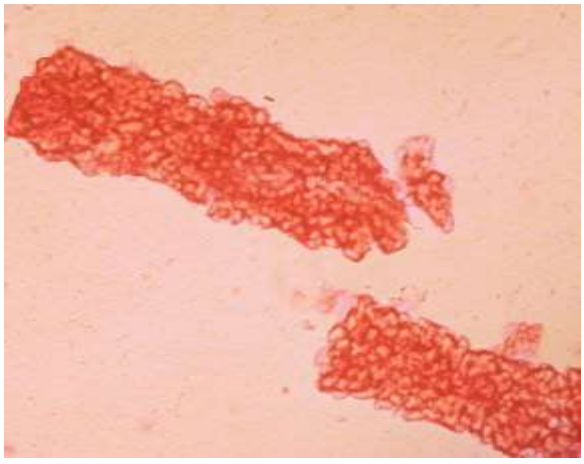
Epithelial cells



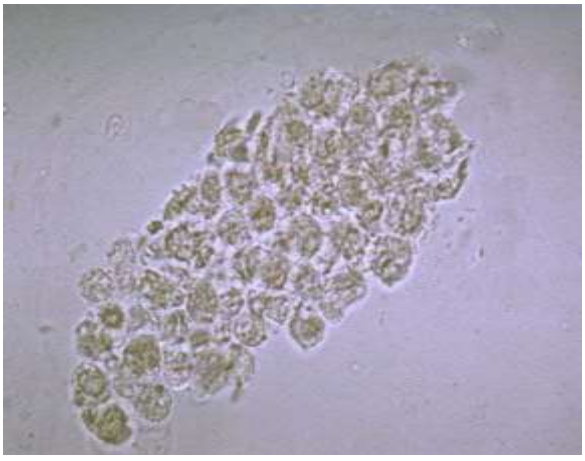
Hyaline cast



Granular cast



Red blood cell cast



White blood cell cast

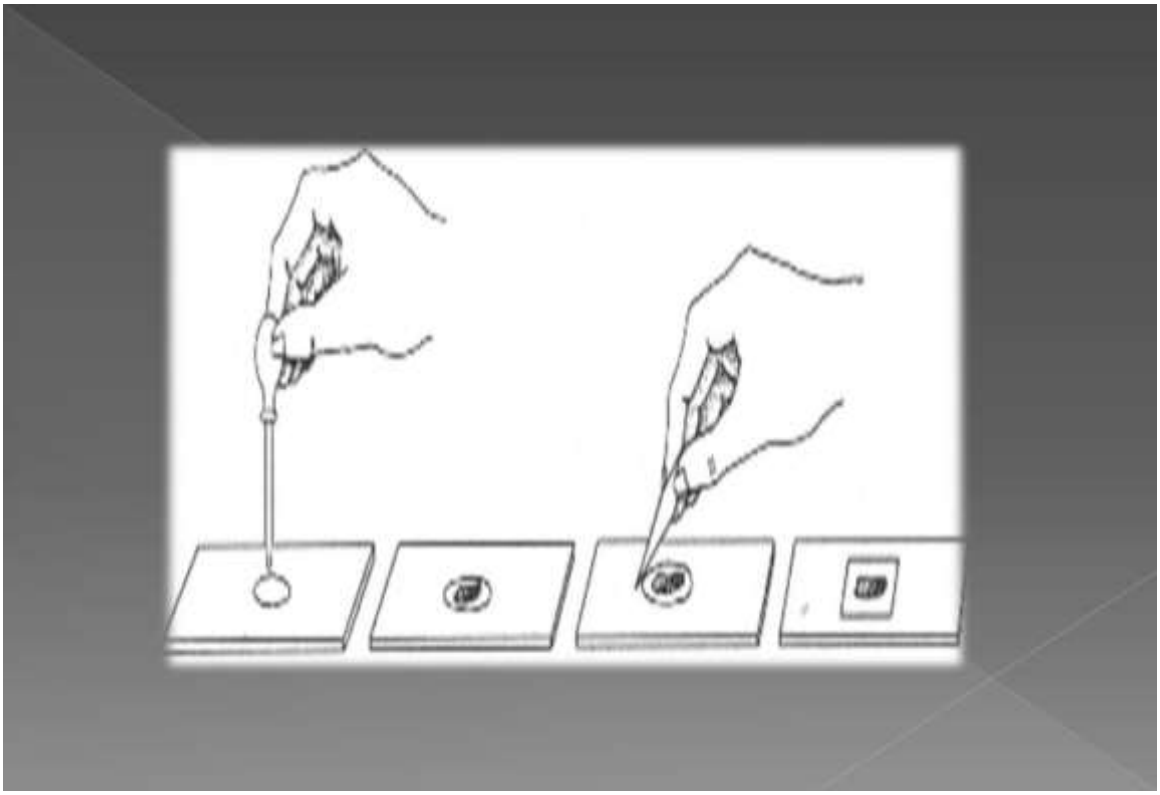
3- Stool:

1- Place a drop of normal saline on a clean slide.

2- Place a small piece of stool on the slide and mix with normal saline, cover with a cover slip. If the specimen contain mucus, the examination prefer to be done without saline. The mucus is put on the slide and covered with cover slip.

3- Examine under 10X and 40X objectives.

4- Report the presence of large numbers of pus cells, RBCs, WBCs, amoebas, flagellates, eggs, larvae, trophozoite and cysts.



* Normal saline (0.85%): is used for routine examination of stool samples, as it is isotonic. It is used to detect worms (eggs and larvae), protozoa (cysts and trophozoite). In addition, it can reveal the presence of RBCs and WBCs.

* Iodine: is used to examine the nuclei of cysts.

* Eosin 1%: is used to provide a pink background and that will help to clear the unstained object.

الأسبوع الخامس

الهدف التعليمي: التعرف على شكل المستعمرة.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة:

Use of Colonial Morphology for the Presumptive Identification of Microorganisms

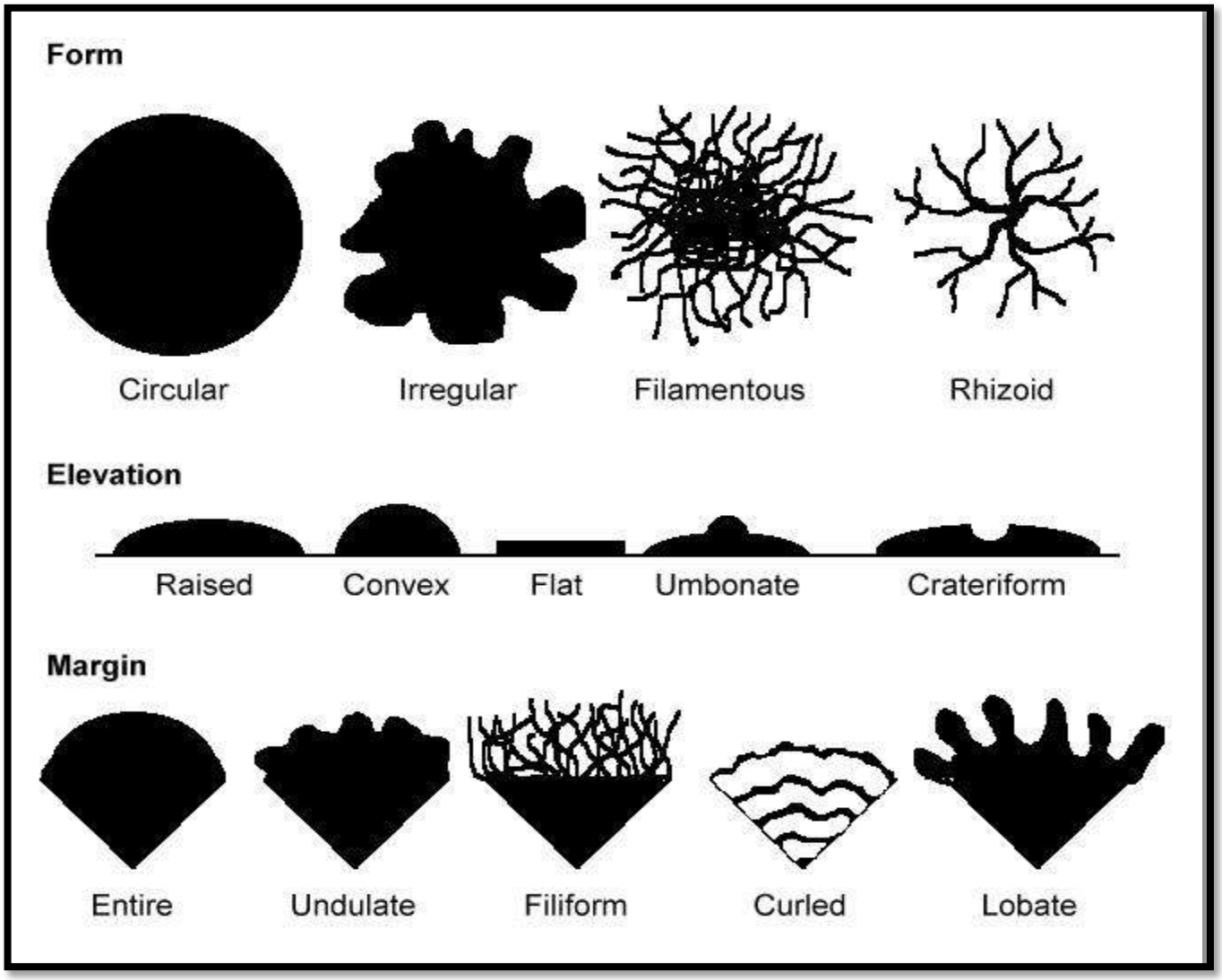
Bacteria:

Colonial morphology are important observations in the primary identification of bacteria. Colonial characteristics include amount of growth and description, type and pattern of hemolysis on blood agar, elevation, margin, surface, consistency and size of the colony.

Table 1: Terms used in colonial morphology of bacteria

Term	Description
Color	By reflected or transmitted light: fluorescent, iridescent, opalescent. **Note:** There are many colors ranging from white to yellow, pink, orange, redor purple.
Pigmentation	Some organisms produce a pigmented colony which is usually enhanced at room temperature, this can be seen on the topside and reverse side of the colony. For example, *Pseudomonas aeruginosa* green pigment and, *Serratia marcescens* red pigment, although non-pigmented strains within a species may occur.
Consistency (texture)	Butyrous (buttery), fluffy, mucoid (thick, stringy, and wet), friable, membranous, rugose (wrinkled), dry, moist, brittle, viscous, powdery, velvety,glabrous, granular, floccose.
Edge/margin	Entire, undulate, lobate, crenated, erose, fimbriate, effuse, filiform, curled,wavy.
Elevation (topography)	Flat, raised, low convex, convex or dome-shaped, umbonate, with or without beveled margin, pulvinate, crateriform.
Emulsifiability	Easy or difficult, forms homogeneous or granular suspension or remainsmembranous when mixed in a drop of water.
Shape/form	Colonial shape is determined by the edge and thickness of the colony: smooth, filiform, spreading, rhizoid, circular, irregular, filamentous, spindle,punctiform, radiate.
Opacity	Transparent, translucent, opaque.

Size	The diameter is usually measured in millimeters. Colony size varies and it is also described in terms such as pinpoint, small, medium and large.
Structure	Amorphous, granular, filamentous, curled.
Surface	Smooth, glistening, rough (fine, medium or coarsely granular), concentric (ringed), papillate, dull or wrinkled, heaped up, contoured, veined.
Degree of growth	Scanty, moderate or profuse.
Type of hemolysis on blood agar	Alfa (α), beta (β), gama (γ).

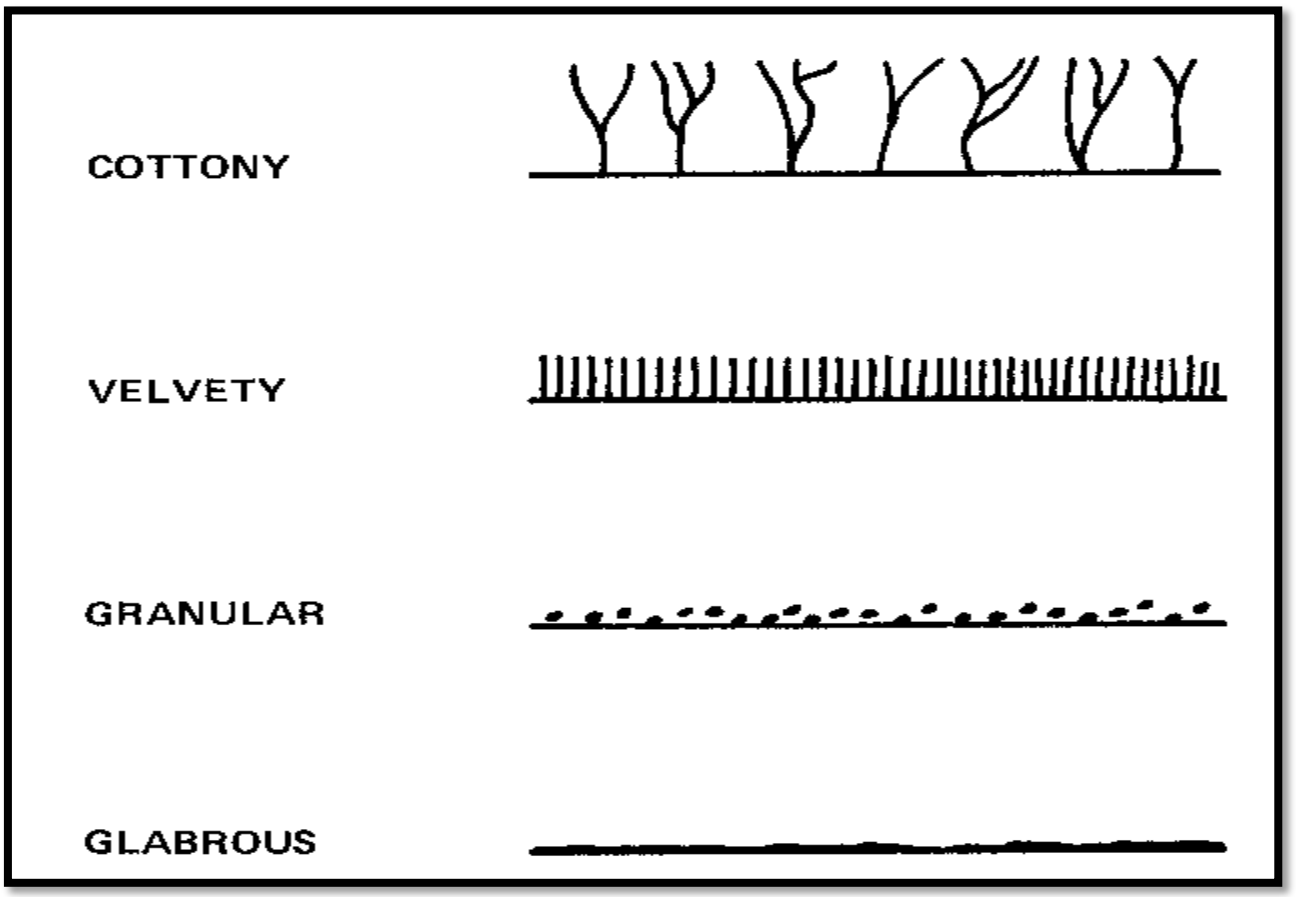


Fungi:

Fungal colonial morphology and growth rate may vary depending on the genus, species, type of culture medium used, age of culture used for subculture, amount of inoculum and the temperature of incubation.

Table 2: Terms used in colonial morphology of yeasts and filamentous fungi

Term	Description
Color	Yeast colonies are usually white, cream, yellow, red, pink or brown. Mold colonies vary greatly, often in shades of green, red, brown or black and the surface color usually reflects the color of the spores. For some groups such as the dermatophytes looking for reverse pigmentation on the underside of colonies can be helpful.
Pigmentation	Pigment production may color the entire colony as with yeast or in some molds it may only be the spores that are pigmented. Colonies of some molds may produce diffusing pigments.
Consistency (texture)	Fungal colony characteristics are dependent upon whether it is yeast or a filamentous fungus. They range from cottony or woolly (floccose), granular, chalky, velvety, powdery, silky, glabrous (smooth), or waxy.
Edge/margin	Entire, undulate, filamentous, lobate, erose (serrated).
Elevation (topography)	Flat, raised, convex, crateriform, heaped, grooved, folded or wrinkled.
Size	The diameter is usually measured in millimeters. Colony size varies and it is also described in terms such as slow-growing, small, medium and large.
Rate of growth	Some fungal colonies are fast growing, covering the entire surface of the agar and taking up all the air-space in a petri-dish whilst other fungi may grow in a restricted manner.



Colonial morphology (Texture)

الأسبوع السادس

الهدف التعليمي: التعرف على اختبارات التشخيص البايوكيميائية.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Biochemical Identification of Bacteria**

Biochemical identification of bacteria involves using specific biochemical tests to determine the presence of certain enzymes or metabolic pathways within bacterial cells . These tests help identify bacteria by analyzing their unique biochemical characteristics , such as their ability to utilize specific substrates , produce certain end products , or express particular enzymes.

1- Catalase Test:

To determine the ability of bacteria that produces catalase enzyme which degrades the hydrogen peroxide . In aerobic organisms , during aerobic respiration , oxygen serves as hydrogen acceptor and hydrogen peroxide is formed in the cell. High concentration of H_2O_2 is formed which is toxic to cell . Bacteria possess the catalase enzyme converts hydrogen peroxide into oxygen and water.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Procedure:

Catalase production can be determined by addition of the substrate H_2O_2 on bacterial culture.

a. Slide method:

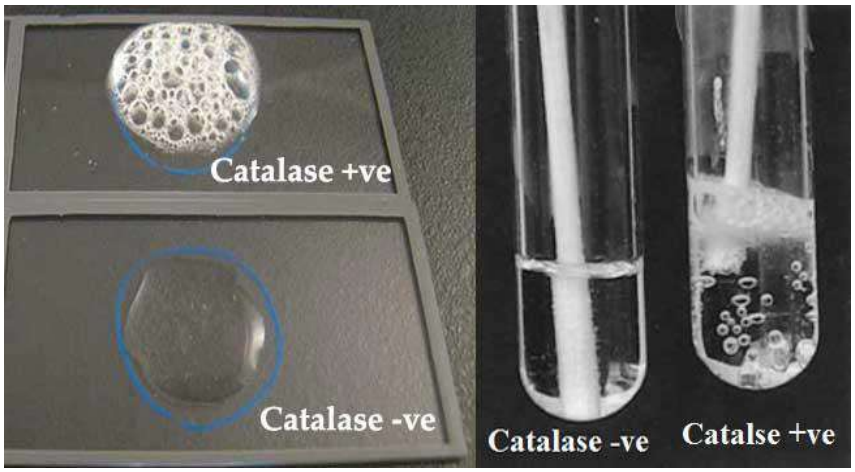
- Pure growth of the organisms will transfer to the clean slide by using inoculation loop or glass rod.
- Immediately add a drop of 3% hydrogen peroxide on bacterial culture.
- Observe the bubble formation.

b. Tube method:

- Take one ml of 3 % hydrogen peroxide in test tube.

- Small amount of bacterial culture introduce into the solution

- Immediately observe the effervescence.

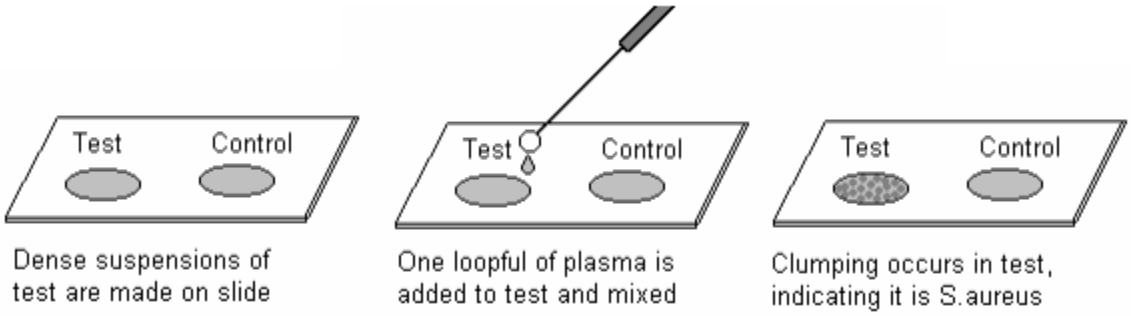


2- Coagulase Test:

Staphylococcus aureus is known to produce coagulase, which can clot plasma into gel in tube or agglutinate cocci in slide. This test is useful in differentiating *S. aureus* from other coagulase-negative staphylococci. Most strains of *S. aureus* produce two types of coagulase, free coagulase and bound coagulase. While free coagulase is an enzyme that is secreted extracellularly, bound coagulase is a cell wall associated protein. Free coagulase is detected in tube coagulase test and bound coagulase is detected in slide coagulase test. Slide coagulase test may be used to screen isolates of *S. aureus* and tube coagulase may be used for confirmation.

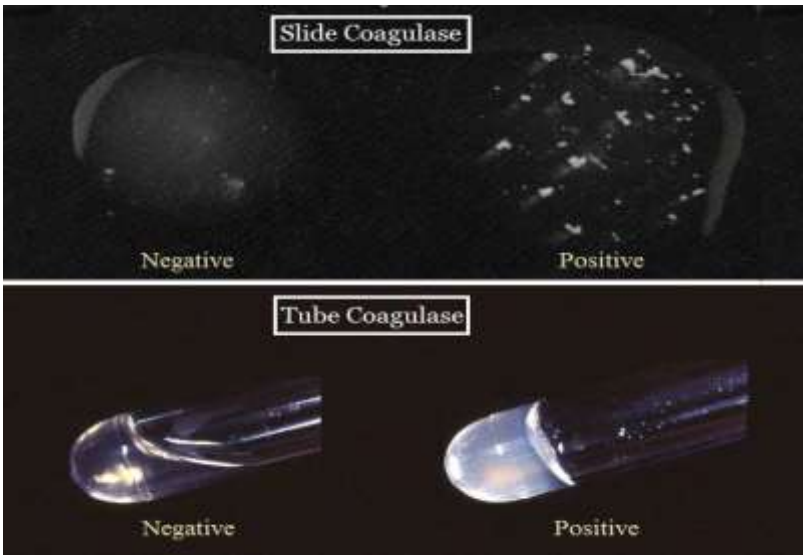
Procedure:

a. Slide method:



b. Tube method:

5 ml of the diluted plasma (Add 0.2 ml plasma in 1.8 ml saline)added to a test tube. About 5 drops of the test organism culture are added to the test tube. The test tube is mixed and incubated at 37°C for an hour. The tube is finally observed for the clot formation. If no clotting is observed, the tube should be examined at 30 minutes interval of up to 6 hours.



3- Oxidase Test:

Oxidase enzymes have an important role in the electron transport system during aerobic respiration. This test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This test is used in the differentiation between the oxidase positive bacteria (*Neisseria*, *Pseudomonas* and *Vibrio*) and the other gram-negative bacteria.

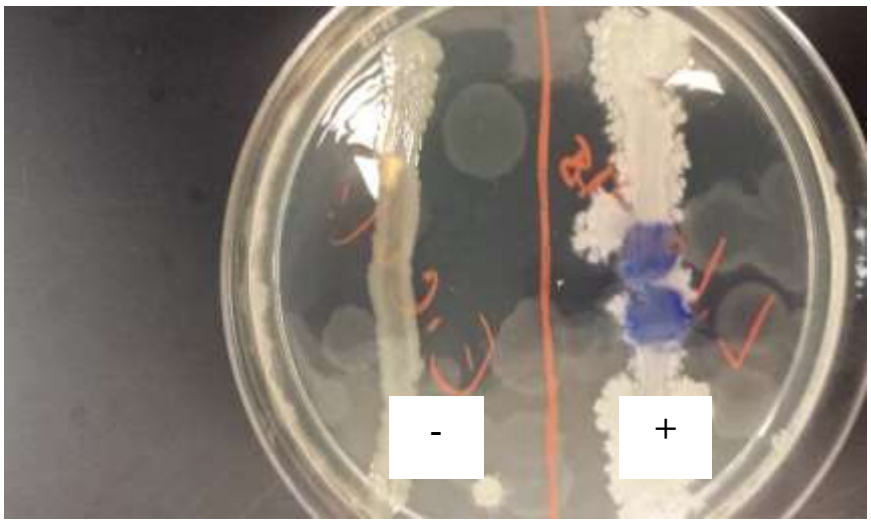
Procedure:

1. Divide the agar plate in two parts by wax pencil.
2. Using a septic technique, streak one part with *Neisseria* or *Pseudomonas*, and streak the other part with *E. coli*.
3. Incubate the plate in an inverted position for 18 to 24 hours at 37ºC.

4. Add 2 to 3 drops of the oxidase reagent to the surface of the growth, on each part.

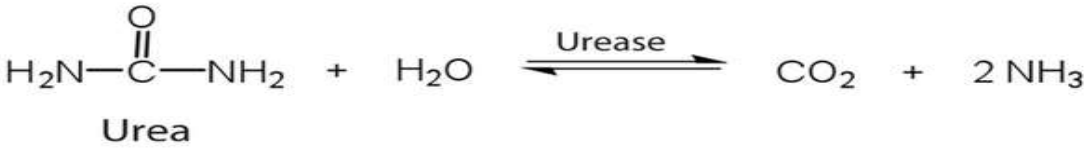
5. Observe the presence oxidase (+) or absence oxidase (-) of color change of colony from pink to purple and finally to dark purple. Color change will occur within 20 to 30 seconds.

6. Record the results.



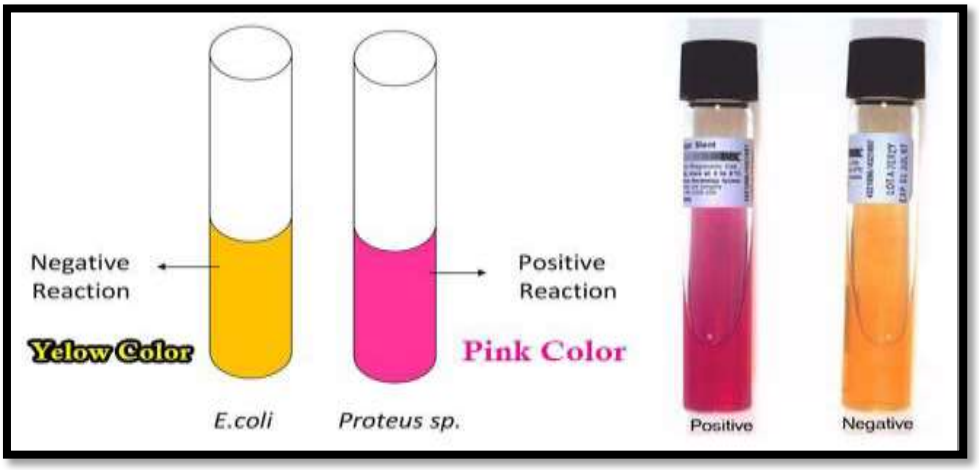
4- Urease Test:

Some bacteria such as Proteus, Klebsiella, Pseudomonas are able to produce an enzyme called urease that hydrolyses urea, forming the end products ammonia, CO_2 and water as follows:



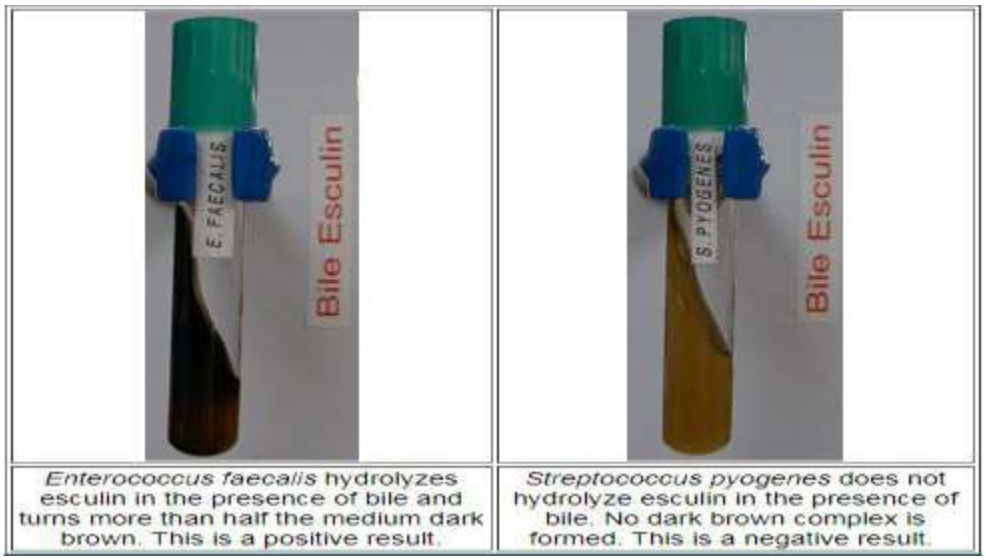
Procedure:

1. Label two urea agar or broth with the name of the bacterium to be tested.
2. Using aseptic technique, inoculate one medium with the bacterium Proteus and the other medium with the bacterium *E. coli*.
3. Incubate at 37ºC for 4 hours or more.
4. Examine change in color and record the results.



5- Bile-Esculin Test:

Bile-esculin test is based on the ability of the group D streptococci and *Enterococcus* species, to hydrolyze esculin in the presence of bile. Bile esculin agar contains bile salts to inhibit the growth of gram positive organisms other than enterococci and group D streptococci. It also contains nutrients, esculin, and ferric citrate. When an organism hydrolyzes the glycoside esculin to form esculetin and dextrose, the esculetin reacts with the ferric citrate to produce a dark brown or black phenolic iron complex.



6- Gelatinase Hydrolysis (Gelatin Liquefaction) Test:

This test is used to determine the ability of an organism to produce extracellular proteolytic enzymes, gelatinases that hydrolyze gelatin. The reaction occurs in two sequential steps: in first reaction gelatinases hydrolyze gelatin into polypeptides and then polypeptides are

further converted into amino acids The amino acids are taken up by the cell and used for metabolic purposes.

Procedure:

1. Label two nutrient gelatin tubes.
2. Using aseptic technique, inoculate (stab) one of the tube with *S. aureus* or *P. vulgaris* and the other tube with *E. coli.*
3. Incubate the tubes for 24 hours or more at 37ºC.
4. Read and record the results after refrigeration.

7- TSIA Test:

The triple sugar iron agar (TSIA) test is a biochemical test used to differentiate bacteria based on their ability to ferment three sugars (glucose, lactose, and sucrose) and release acid and hydrogen sulfide (H_2S) gas which reacts with ferric ions in the medium to produce iron sulfide (black insoluble precipitate).

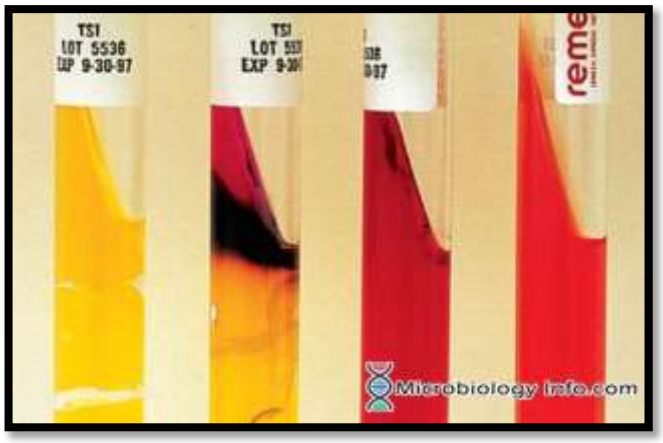
Procedure:

1. Touch a well-isolated colony from a fresh culture of the test bacterium that is 18 to 24 hours old using a sterile inoculating wire.

2. Stab the bottom up to 3 to 5 mm above the base of the test tube using the inoculating wire and while withdrawing, streak the slant.

3. Incubate the tube aerobically (with a loose cap) at 35±22°C for about 24 hours.

4. Examine for color change of the slant and bottom and report the color within 24 hours of incubation. (If you want to read the H_2S production, incubate it for another 24 to 48 hours, but read sugar fermentation and color change within the first 24 hours of inoculation and incubation).



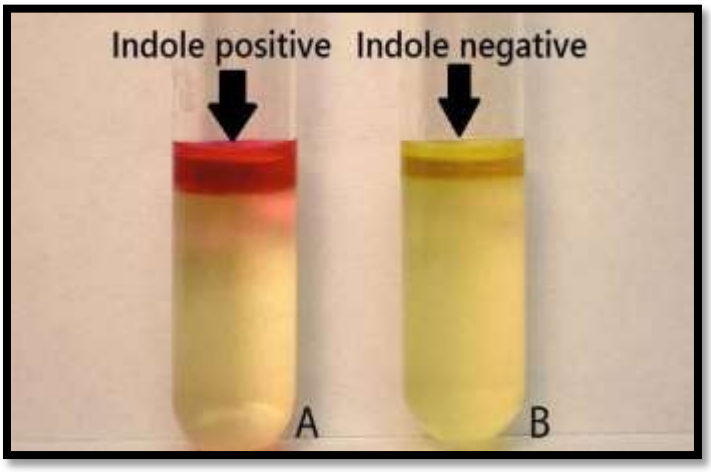
Triple sugar iron agar tubes (from the left):
1- Acid slant/acid bottom with gas, no H_2S (A/A).
2- Alkaline slant/acid butt, no gas, H_2S-positive (K/A H2S+).
3- Alkaline slant/alkaline butt, no gas, no H_2S (K/K).
4- Uninoculated tube (negative control).

8- IMViC Tests:

Each of the letters in "IMViC" represent one of four tests; "**I"** is for indole; "**M**" is for methyl red; **"V"** is for Voges-Proskauer and **"C"** is for citrate utilization. Lowercase **"i"** is added for the ease of pronunciation. IMViC tests are employed in the identification / differentiation of members of family enterobacteriaceae.

Indole Test:

It is performed in tryptophan broth (peptone water). It is used to determine the ability of bacteria to produce the enzyme tryptophanase. Tryptophanase breakdown tryptophan to release indole. Tryptophan is an amino acid that can suffer deamination and hydrolysis by bacteria that express tryptophanase enzyme.

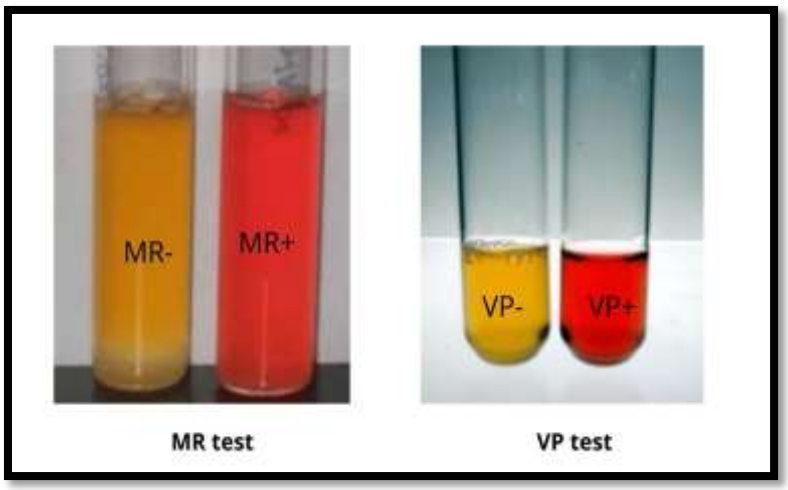


Methyl Red Test:

Some bacteria have the ability to utilize glucose and convert it to a stable acid like lactic, acetic or formic acid as the end product. These bacteria initially metabolize the glucose to pyruvic acid , which is further metabolized through the mixed acid pathway to produce the stable acid **(**glucose is fermented and produces several organic acids "lactic, acetic and formic acids"). The type of acid produced differs from species to species and depends on the specific enzymatic pathways present in the bacteria. 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.

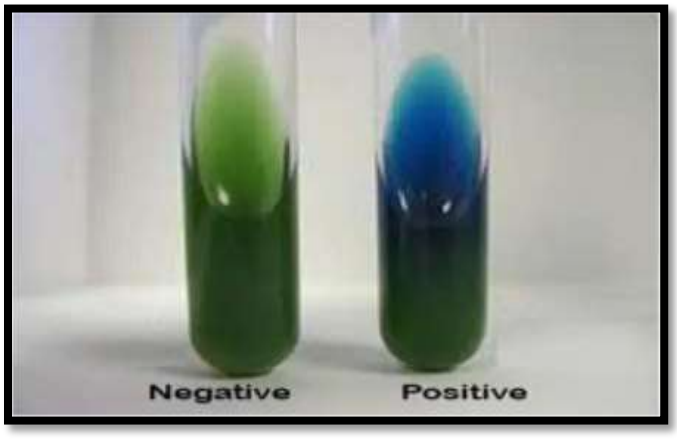
Voges-Proskauer Test:

This test done to determine the ability of the bacteria to produce a neutral end product acetylmethylcarbinol (acetone) from glucose fermentation (red color) . If the culture is negative for acetone, it will remain yellow.



Citrate Utilization Test:

It is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic.



الأسبوع السابع

الهدف التعليمي: التعرف على الطرق المناعية لتشخيص الأحياء المجهرية.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Immunological Methods used for Microorganism Detection**

Immunological methods utilize the specific binding of antibodies to antigens for microorganism detection. These methods exploit the body's natural immune response to identify and quantify microorganisms or their components. Key techniques include immunoassays like ELISA, immunofluorescence, agglutination, and precipitation tests.

1- Immunoassays:

a- Enzyme-Linked Immunosorbent Assay (ELISA): this widely used method uses enzyme-labeled antibodies to detect the presence of antigens or antibodies in a sample. It's known for its sensitivity and suitability for large-scale testing.

b- Immunofluorescence: this technique uses fluorescently labeled antibodies to bind to specific antigens on microorganisms, allowing them to be visualized under a fluorescence microscope.

c- Western Blotting: this method separates proteins (including antigens) by size and then uses antibodies to detect specific proteins of interest.

d- Lateral Flow Immunoassay: this rapid, point-of-care test uses antibody-antigen binding to produce a visible result, often in the form of a colored line.

2- Agglutination and Precipitation:

a- Agglutination: this method involves antibodies causing microorganisms or antigen-coated particles to clump together (agglutinate).

b- Precipitation: similar to agglutination, but involves soluble antigens forming a precipitate with antibodies.

3- Other Methods:

a- Radioimmunoassay (RIA): this technique uses radioactive labels on antigens to detect antigen-antibody complexes.

b- Immunomagnetic Separation: this method uses magnetic beads coated with antibodies to capture specific microorganisms from a sample.

c- Flow Cytometry: this technique allows for the rapid analysis of multiple characteristics of individual cells, including the presence of specific antigens, by using labeled antibodies.

الأسبوع الثامن

الهدف التعليمي: التعرف على تقنية تهجين الحامض النووي.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Applications of Molecular Diagnostics**

Nucleic Acid Hybridization Techniques:

Nucleic acid hybridization is a molecular biology technique used to identify specific DNA or RNA sequences within a sample. It involves the pairing of complementary single-stranded nucleic acid molecules (DNA or RNA) to form double-stranded molecules, or hybrids. This process allows researchers to detect and analyze the presence and abundance of particular nucleic acid sequences.

Principle:

1- Denaturation: the target nucleic acid (DNA or RNA) and a labeled probe (a short, single-stranded DNA or RNA sequence complementary to the target) are separated into single strands, typically by heating.

2- Hybridization: the denatured probe and target are mixed under conditions that allow complementary sequences to bind to each other.

3- Detection: the labeled probe allows for the detection and quantification of the target sequence.

Types of Hybridization Techniques:

Southern blotting: used to identify specific DNA sequences in a sample after separation by gel electrophoresis.

Northern blotting: used to identify specific RNA sequences after separation by gel electrophoresis.

In situ hybridization: used to identify nucleic acid sequences within cells or tissues

Applications:

Nucleic acid hybridization is a versatile technique with numerous applications, including:

Gene identification and characterization: identifying specific genes and their variations.

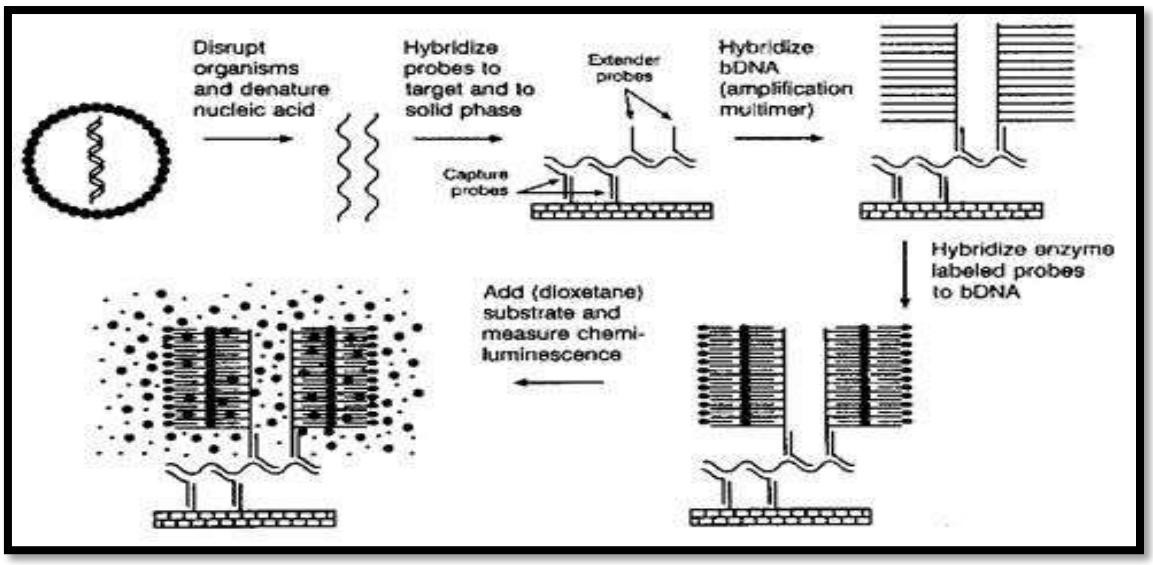
Detection of pathogens: detecting the presence of viruses , bacteria , or other micro-organisms.

Diagnosis of genetic diseases: Identifying mutations associated with genetic disorders.

Cancer research: Studying gene expression patterns in cancer cells.

Forensic science: Identifying individuals based on their DNA.

Development of new drugs and therapies: Identifying potential drug targets.



الأسبوع التاسع

الهدف التعليمي: التعرف على طريقة تضخيم الحامض النووي.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Nucleic Acid Amplification Procedures**

Nucleic acid amplification procedures are techniques used to create multiple copies of a specific DNA or RNA sequence, enabling detection and analysis of even minute amounts of genetic material. These procedures are crucial in various fields like molecular diagnostics, research, and forensics.

Key Concepts and Processes:

Target Amplification: the core principle involves amplifying a specific target sequence within a larger nucleic acid sample.

Enzymatic Replication: enzymes , like DNA polymerases, are used to synthesize new DNA or RNA strands complementary to the target sequence.

Exponential Amplification: with repeated cycles of amplification, the target sequence can be exponentially increased, resulting in a large number of copies.

Common Amplification Techniques:

Polymerase Chain Reaction (PCR): a widely used method involving repeated cycles of denaturation, annealing, and extension to amplify DNA sequences.

Transcription-Mediated Amplification: utilizes an RNA polymerase to create RNA copies from a DNA template.

Strand Displacement Amplification: employs a DNA polymerase that can displace the previously synthesized strand to allow for continuous amplification.

Loop-Mediated Isothermal Amplification (LAMP): a method that amplifies DNA under isothermal conditions (constant temperature) using a DNA polymerase and a set of specifically designed primers.

Recombinase Polymerase Amplification (RPA): an isothermal amplification method that uses recombinase enzymes to facilitate primer binding and DNA synthesis.

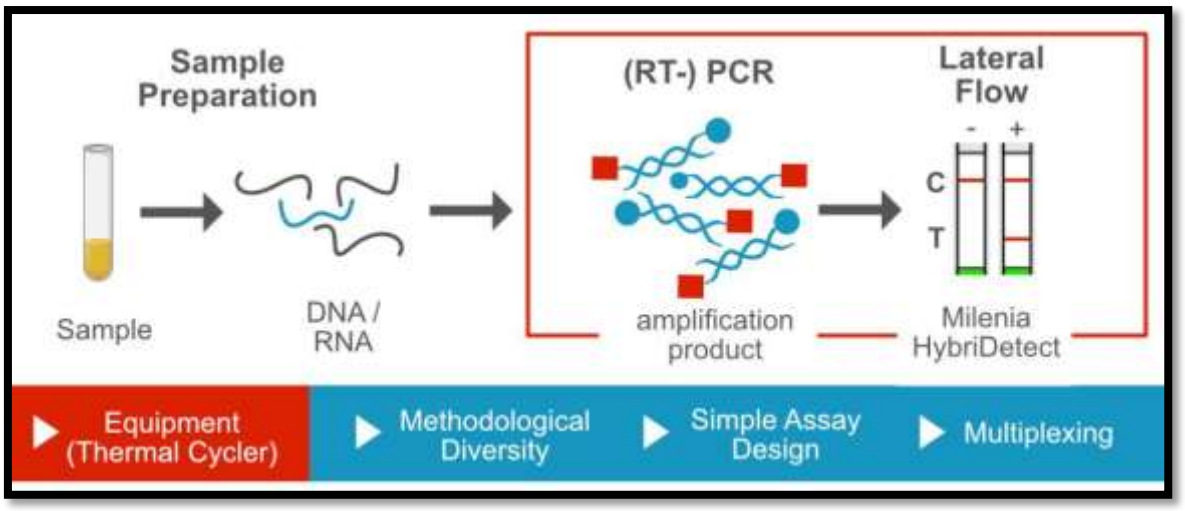
Applications:

Diagnostics: detecting pathogens (viruses , bacteria) , genetic diseases , and monitoring treatment responses.

Research: studying gene expression, gene cloning, and other molecular biology experiments.

Forensics: DNA fingerprinting and identification of individuals.

Environmental Monitoring: detecting and quantifying microorganisms in various environments.



الأسبوع العاشر

الهدف التعليمي: التعرف على تسلسل الحامض النووي المعتمد على التضخيم.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Nucleic Acid Sequence Based Amplification**

Nucleic Acid Sequence-Based Amplification (NASBA) is an isothermal, in vitro nucleic acid amplification technique that amplifies RNA and DNA targets as single-stranded RNA. It's a primer-dependent method that utilizes enzymes like reverse transcriptase, RNase H, and T7 RNA polymerase to achieve exponential amplification at a constant temperature. NASBA is particularly known for its ability to amplify RNA targets, making it a valuable tool in RNA detection and analysis.

Isothermal Amplification: NASBA is an isothermal process, meaning it amplifies nucleic acids at a single, constant temperature, unlike PCR which requires thermal cycling.

Enzyme Cocktail: NASBA relies on a cocktail of enzymes: reverse transcriptase, RNase H, and T7 RNA polymerase. These enzymes work in a coordinated manner to amplify the target sequence.

Primer Dependence: NASBA requires the use of specific oligonucleotide primers that bind to the target RNA or DNA sequence.

RNA Amplification: NASBA is particularly useful for amplifying RNA targets. It can be used to detect RNA viruses, bacteria, and other pathogens.

Mechanism:

The process involves reverse transcription of RNA into cDNA, followed by transcription of the cDNA into multiple copies of RNA, all occurring at a single temperature

Applications:

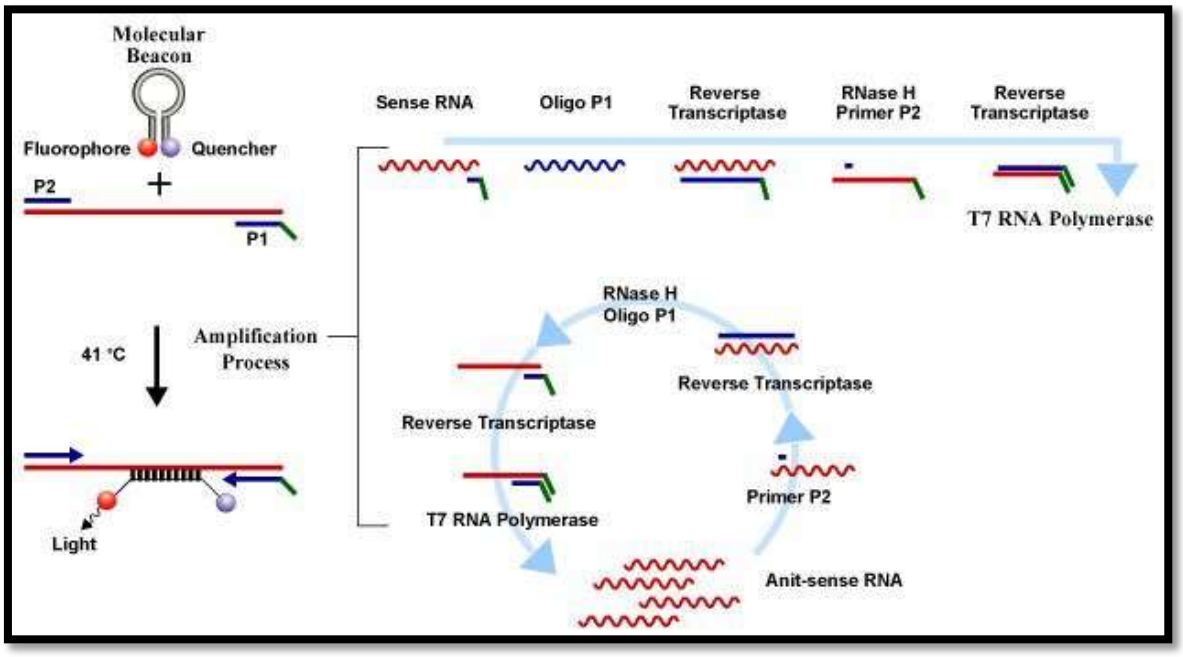
NASBA is used in various applications, including:

- **Detection of RNA viruses:** NASBA is a sensitive method for detecting RNA viruses like HIV, CMV, and influenza.

- **Detection of bacterial RNA:** It can be used to identify and quantify bacterial RNA in clinical samples

- **SNP analysis:** NASBA can be combined with other techniques like molecular beacons for single nucleotide polymorphism (SNP) analysis

- **Gene expression studies:** NASBA can be used to quantify gene expression levels.



الأسبوع الحادي عشر

الهدف التعليمي: التعرف على اختبار الحساسية الدوائية.

مدة المحاضرة:4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility testing (AST), often referred to as antibiotic susceptibility testing, is a crucial laboratory procedure used to determine which antibiotics effectively inhibit the growth of a specific microorganism (bacteria or fungi) causing an infection. This testing helps guide clinicians in selecting the most effective antibiotics for treating a patient's infection and also monitors the development of antibiotic resistance.

How it's done:

1- Microorganism isolation: the first step is to isolate the microorganism (bacteria or fungus) from the patient's sample (e.g., blood, urine, wound tissue).

2- Exposure to antibiotics: the isolated microorganism is exposed to different antibiotics in a controlled laboratory setting, using various methods.

3- Observation and interpretation: the laboratory observes the growth of the microorganism in the presence of each antibiotic and interprets the results based on established breakpoints. These breakpoints categorize the microorganism as susceptible (sensitive), intermediate (moderately sensitive), or resistant to the antibiotic.

Common Methods used in AST:

- Disk diffusion (Kirby-Bauer): antibiotic-impregnated paper disks are placed on an agar plate inoculated with the microorganism. The size of the zone of inhibition (area around the disk where bacteria do not grow) indicates susceptibility.

- Broth microdilution: the microorganism is grown in a series of dilutions of different antibiotics in liquid medium. The lowest concentration of antibiotic that inhibits visible growth (MIC) is determined.

- Etest (gradient diffusion): a plastic strip containing a concentration gradient of an antibiotic is placed on an agar plate inoculated with the microorganism. The intersection of the zone of inhibition with the strip indicates the MIC.

Selecting Antimicrobial Agents for Testing:

Selecting antimicrobial agents for testing involves carefully considering several factors to ensure relevant and effective testing. The primary factors include the likely infecting microorganism, its usual susceptibility profile, the site of infection, and the institution's formulary and antibiotic usage patterns.

Key Considerations for Selection:

1- Infecting Microorganism: identify the most probable microorganisms based on the site of infection (e.g., Gram-positive bacteria in skin and soft tissue infections, Gram-negative Consider the patient's clinical history, including previous bacteria in urinary tract infections). infections and colonization with resistant organisms.

2- Susceptibility Profile: assess the known susceptibility patterns of the suspected Use institutional antibiograms to guide the selection pathogens in the local area or hospital. of antimicrobial agents for testing.

3- Site of Infection: choose agents that can effectively reach the site of infection and achieve Factors like blood-brain barrier adequate concentrations to inhibit or kill the pathogen. penetration, tissue penetration, and potential for inactivation need to be considered.

4- Institutional Formulary and Usage: prioritize testing of antimicrobial agents that are Align testing with prescribing practices to frequently used in the institution's formulary. ensure relevant data for clinical decision-making.

5- Prophylactic Use: for surgical or invasive procedures, select agents based on the type of procedure and the anticipated pathogens.

Use local antibiograms to refine prophylactic choices and ensure coverage of likely pathogens.

6- Method Selection: consider the available methods for antimicrobial susceptibility testing (e.g., disk diffusion, broth microdilution) and choose methods appropriate for the organism and the antimicrobial agents being tested.

Reporting Antimicrobial Susceptibility Test (AST) Results:

Reporting antimicrobial susceptibility test (AST) results is crucial for guiding appropriate antibiotic therapy. Results are typically reported as susceptible (S), intermediate (I), or resistant (R), often with a minimum inhibitory concentration (MIC) value for more quantitative results. Selective reporting, where only certain antibiotics are reported, is a strategy used to optimize antibiotic use and reduce the prescription of broad-spectrum agents.

Key Aspects of Reporting AST Results:

Qualitative vs. Quantitative: AST results can be qualitative (S, I, R) or quantitative (MIC).

MIC Determination: the MIC is the lowest concentration of an antimicrobial that inhibits visible growth of a microorganism.

Selective Reporting: this involves reporting results for a limited set of antibiotics, often prioritizing first-line and narrow-spectrum agents.

Cascade Reporting: a type of selective reporting where results for secondary agents are only reported if the organism is resistant to primary agents.

Optimization: strategies like selective reporting and timely communication of results are crucial for optimizing antimicrobial stewardship and improving patient outcomes.

الأسبوع الثاني عشر

الهدف التعليمي: التعرف على الطرق التقليدية لاختبار الحساسية المضادة للميكروبات.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Traditional Antimicrobial Susceptibility Testing Methods**

Inoculum Preparation and Use of McFarland Standards:

Inoculum preparation for antimicrobial susceptibility testing involves creating a standardized suspension of bacteria to ensure reliable and reproducible results. This is typically achieved by adjusting the turbidity of a bacterial broth or suspension to match a specific standard, such as the 0.5 McFarland standard, or by using devices that deliver a known number of bacteria.

Procedure:

1. Preparing the Bacterial Suspension:

- From Colonies: typically, a few colonies (e.g., 4-5) of a pure bacterial culture are picked with a sterile loop and suspended in a suitable broth (e.g., Mueller-Hinton broth).

- Direct from Agar Plate (Optional): some methods allow for inoculum preparation directly from agar plates, using devices like the Inoclic rod or a similar wand, which removes a reproducible number of bacteria.

2. Standardizing the Inoculum:

- Turbidity Standards: the most common method involves adjusting the turbidity of the bacterial suspension to match a 0.5 McFarland standard (or its equivalent). This ensures a consistent bacterial concentration (typically 1-2 x 10^8 CFU/ml).

- Specialized Devices: devices like the rapid inoculum standardization system or the 3M system offer alternative ways to standardize the inoculum by delivering a known number of bacteria.

3. Application for Different Tests:

- Disk Diffusion: a standardized swab (e.g., from a 0.5 McFarland standard suspension) is used to uniformly streak the agar plate. Antibiotic disks are then placed on the agar surface, and the plates are incubated.

- Broth Microdilution/Macrodilution: bacterial suspensions are diluted to the desired concentrations and used to inoculate microtiter plates or tubes containing serial dilutions of the antimicrobial agent.

- MIC Determination: the minimum inhibitory concentration (MIC) is determined by identifying the lowest concentration of antimicrobial agent that inhibits visible growth.

Dilution Susceptibility Testing Methods:

Dilution susceptibility testing, a method for determining antimicrobial effectiveness, involves exposing microorganisms to a range of antimicrobial concentrations, either in broth or on agar plates. The goal is to find the minimum inhibitory concentration (MIC), the lowest concentration of the drug that prevents visible growth of the microorganism. There are two main types: broth dilution (including macro- and microdilution) and agar dilution.

1. Broth Dilution:

- Broth Macrodilution: this method involves preparing serial dilutions of the antimicrobial agent in liquid broth, typically in test tubes. A standardized microbial suspension is then added to each tube, and after incubation, the MIC is determined by identifying the lowest concentration that inhibits visible growth.

- Broth Microdilution: similar to macrodilution, but utilizes smaller volumes in 96-well microplates, allowing for testing multiple dilutions and organisms simultaneously. This is a common reference method, particularly for bacteria that grow aerobically.

2. Agar Dilution: this method involves incorporating antimicrobial agents into agar medium at varying concentrations. A standardized microbial inoculum is then applied to the agar surface. The MIC is determined by identifying the lowest concentration of the antimicrobial that inhibits visible growth after incubation. Agar dilution is a reference method for several bacterial species, including Bacteroides and Parabacteroides, as well as *N. gonorrhoeae* and *H. pylori*.

Antimicrobial Stock Solutions:

Antimicrobial stock solutions are concentrated solutions of antibiotics or other antimicrobial agents used in laboratory settings to inhibit or kill microorganisms. These solutions are typically prepared at a higher concentration than what is used in experiments (e.g., 1000x) and are then diluted to the desired working concentration. Common examples include ampicillin, kanamycin, chloramphenicol, and tetracycline.

Examples of Antimicrobial Stock Solutions:

Ampicillin: often prepared as a 100 mg/ml stock solution in sterile water.

Chloramphenicol: can be prepared as a 25 mg/ml stock solution in 100% ethanol.

Kanamycin: a 50 mg/ml stock solution in sterile water is common.

Tetracycline: a 10 mg/ml stock solution in sterile water is used, but it is light-sensitive and should be protected from light.

Rifampicin: a 16 mg/ml stock solution in methanol is common, but it is light-sensitive and toxic.

الأسبوع الثالث عشر

الهدف التعليمي: التعرف على اختبار نشر القرص.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Disk diffusion testing**

Disk diffusion testing, also known as the Kirby-Bauer test, is a method used to determine the susceptibility of bacteria to various antimicrobial agents. It involves inoculating a petri dish with bacteria and placing antibiotic-impregnated paper disks on the agar surface. The resulting zones of inhibition (areas where bacterial growth is inhibited) are measured and interpreted to categorize the bacteria as susceptible, intermediate, or resistant to the antibiotics.

Principle:

The core principle is that antibiotics diffuse from the disk into the surrounding agar, creating a concentration gradient. Bacteria exposed to a high enough concentration of a suitable antibiotic will be inhibited or killed, leading to a zone of inhibition around the disk.

Establishing Zone Diameters:

The diameter of the zone of inhibition is measured to the nearest millimeter using a ruler, caliper, or automated zone reader. The measurement is taken from the back of the plate (for unsupplemented Mueller-Hinton agar) or from the front (with the lid removed for MH-F agar) using reflected light against a dark background. The measurement is taken at the point where no obvious growth is detected by the naked eye.

Interpretive Breakpoints:

Zone diameters are compared to established interpretive breakpoints (ranges of zone sizes that indicate susceptibility, intermediate resistance, or resistance).

These breakpoints are determined by correlating zone sizes with minimum inhibitory concentrations (MICs) and clinical outcomes , and are specific to each bacterial species and antibiotic combination.

The clinical and laboratory standards institute (CLSI) and the European committee on antimicrobial susceptibility testing (EUCAST) are major organizations that provide standardized breakpoints for disk diffusion testing.

Disk Storage:

- Proper storage of antibiotic disks is crucial to maintain their potency.
- Disks should be stored in a refrigerator (2-8°C) or freezer , depending on the specific recommendations for the antibiotic.
- Desiccants are used to prevent moisture from affecting the disks.
- Stability testing (accelerated and real-time) is performed to determine expiration dates.

Inoculation and Incubation:

- A standardized inoculum of the bacteria is prepared (usually a 0.5 McFarland standard).
- The inoculum is spread evenly over the surface of a Mueller-Hinton agar plate.
- Antibiotic disks are applied to the agar surface within 15 minutes of inoculation.
- Plates are incubated at a specified temperature (usually 35°C) and atmospheric conditions (e.g., ambient air).

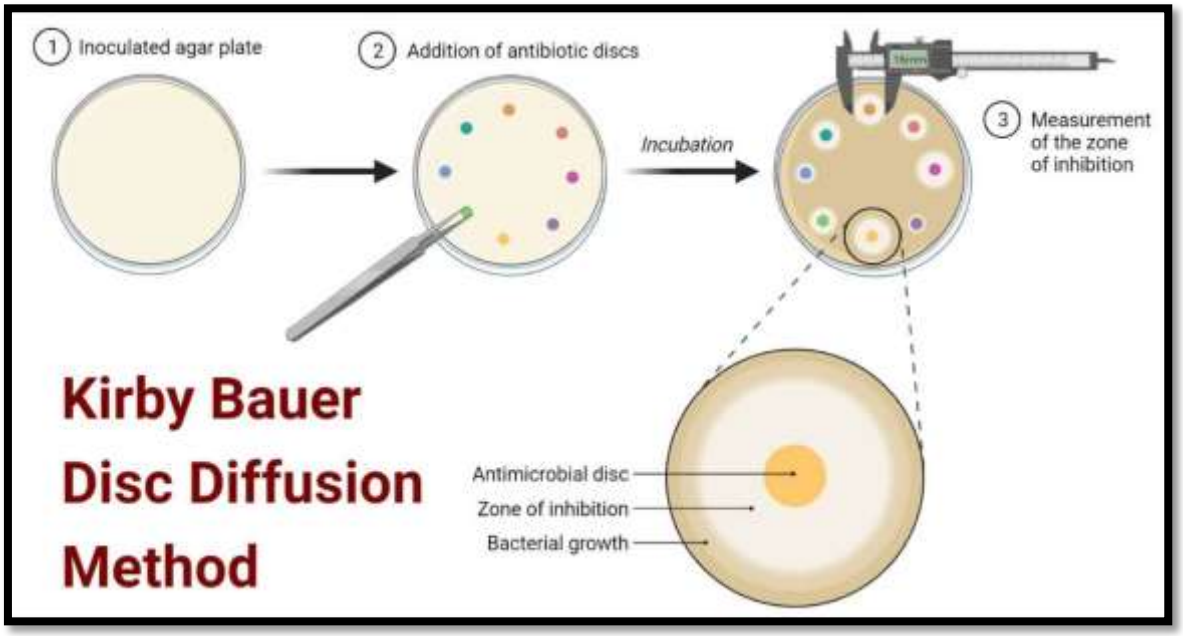
Reading Plates and Test Interpretation:

- After incubation, zones of inhibition are examined and measured.

- The zone diameters are interpreted using the appropriate interpretive breakpoints (e.g., CLSI or EUCAST).

- The bacteria are categorized as susceptible (S) , intermediate (I) , or resistant (R) to the antibiotic.

Colonies within the zone of inhibition may indicate resistance, but this should be interpreted carefully as some organisms have natural resistance mechanisms or may exhibit </double zones/> (e.g. hemolytic streptococci on MH-F agar).



الأسبوع الرابع عشر

الهدف التعليمي: التعرف على الطرق المحورة لاختبار البكتريا ذات النمو البطيء أو ذات المتطلبات الخاصة.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: Modified Methods for Testing Slow-Growing or Fastidious Bacteria

Modified methods for testing slow-growing or fastidious bacteria often involve adapting traditional techniques like disk diffusion or broth microdilution to accommodate their specific growth requirements, or utilizing newer, faster methods like molecular diagnostics or specialized phenotypic assays.

Modified Techniques:

1- Modified Disk Diffusion: the standard Kirby-Bauer disk diffusion method can be adapted for some fastidious bacteria by using enriched media (like chocolate agar or blood agar) and specialized incubation conditions (e.g., increased CO_2).

2- Modified Broth Microdilution: similar to disk diffusion , broth microdilution can be modified by using enriched media and adjusting incubation times and conditions.

3- Etest: this method utilizes a plastic strip with a concentration gradient of an antibiotic, allowing for the determination of the minimum inhibitory concentration (MIC). It can be adapted for fastidious bacteria by using appropriate media.

4- Specialized Media: enriched media such as blood agar , chocolate agar , or Loeffler's serum slope are crucial for growing and testing fastidious bacteria.

Examples of Bacteria and Modified Methods:

***Haemophilus influenzae*:** requires enriched media like chocolate agar and is often tested using modified disk diffusion or Etest.

Neisseria gonorrhoeae: requires enriched media and specific incubation conditions and is often tested using disk diffusion or broth microdilution.

Streptococcus species: can be tested using modified disk diffusion or broth microdilution.

الأسبوع الخامس عشر

الهدف التعليمي: التعرف على اختبار الحساسية للبكتريا اللاهوائية.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Susceptibility Testing for Anaerobic Bacteria**

Susceptibility testing for anaerobic bacteria is crucial for guiding appropriate antibiotic therapy, as resistance patterns are not always predictable. Methods include agar dilution, broth microdilution, and MIC gradient diffusion methods like Etest. The agar dilution method is the gold standard, while broth microdilution is commonly used for routine testing. Etest is also a useful alternative for individual patient isolates.

Methods for Susceptibility Testing of Anaerobes:

1- Agar Dilution: this is the reference method, involving incorporating antibiotics into agar plates and inoculating with bacteria to determine the minimum inhibitory concentration (MIC).

2- Broth Microdilution: this method uses serial dilutions of antibiotics in broth and is considered less labor-intensive than agar dilution.

3- MIC Gradient Diffusion Method (Etest): this method uses a plastic strip impregnated with a gradient of antibiotic, allowing for direct determination of the MIC from the zone of inhibition.