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

الحقيبة التدريسية لمادة تقنيات مختبرية نظري

الصف: الأول

تدريسي المادة

م.د. رواء صادق مجيد

الفصل الدراسي الأول

جدول مفردات مادة تقنيات مختبرية نظري

Week	Details
1-3	Introduction to Medical lab. Techniques. Identify the various laboratory glasses and how to deal with laboratory methods. Sterilization. Identify ways of cleaning, sterilization and disinfectant by physical, chemical and mechanical means. Identify different sterilization equipment and materials used in chemical sterilization A full review of the basic techniques that use in the diagnosis of bacteria, blood. Laboratory safety and how to avoid accidents and errors that are inadvertently laboratory in a laboratory (first aid, biochemical hazards, and biological hazards), and biological and chemical safety.
4	Samples collection and handling. Samples collection for different lab. Investigations, samples transport, samples preparation.
5	Culturing of microorganism: types of Culture media, different samples used for culture, bacterial growth curve, MO characterization (chemical tests for MO identification)
6	Urine samples: Urine formation, Properties of urine, chemical and physical investigations, microscopic examination.
7	Stool sample: formation, properties, culture, general examination.
8	Seminal Fluid: Formation, organs of reproductive tract, characterization of semen fluid, investigations that used on seminal fluid, seminal fluid examination, fructose test, antisperm antibody (serum and semen). Total sperm count in Neubar chamber. Types of normal and abnormal of Sperms character with study the way of writing the final report.
9	Agglutination technique
10	Advance techniques Enzyme-linked immunosorbent assay (ELISA) principle, applications
11	Radioimmunoassay (RIA) principle, applications
12	Immunofluoresence technique
13	Polymerase chain reaction (PCR), types principle, applications
14	Real-time PCR
15	Review

الهدف من دراسة مادة تقنيات مختبرية (الهدف العام):

تهدف دراسة مادة تقنيات مختبرية للصف الأول إلى:

1- التعرف على المفاهيم العامة للمختبر الطبي.

2- التعرف على التقنيات الضرورية للعمل في المختبر الطبي.

الفئة المستهدفة:

طلبة الصف الأول/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 تقنيات المختبرات الطبية وكذلك التعرف على الأدوات الزجاجية الموجودة في المختبر الطبي والتعرف على بعض التقنيات المستخدمة في المختبرات الطبية مثل التنظيف والتعقيم والتطهير.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Laboratory Techniques:**

Introduction:

Laboratory techniques can be defined as the branch of laboratory medicine which deals with the examination of tissues and excretions of the human body and body fluids by various electronic, chemical, microscopic and other medical laboratory procedures or techniques either manual or automated which will aid the technician in the diagnosis, study and treatment of disease and in the promotion of health in general.

Clinical laboratory science professionals (also called medical laboratory scientists or medical laboratory technicians), are highly skilled scientists who determine the best treatment for the patient.

Departments and Their Functions:

Laboratory techniques can be entered in all areas of the clinical laboratory application which include:

1. Clinical Chemistry:

It is department performs hundreds of quantitative analyses on a variety of body fluids to determine values for chemical components in normal and diseased states. Routine tests run by the clinical chemistry section analyze levels of glucose or hemoglobin A1c, blood urea, lipids, liver function values, enzymes, renal function test, protein, vitamin, and electrolytes.

2. Hematology:

This department performs tests that are important in diagnosing many disorders such as anemia, polycythemia, leukocytosis, leukopenia, thrombocytosis, thrombocytopenia and leukemia. The most common test performed in this department is the complete blood count (CBC), which is a summary of cell counts (i.e., red, white, and platelet), total hemoglobin level, red blood cell size, and hematocrit.

3. Microbiology:

The microbiology department identifies microorganisms that cause disease and determine the most effective antibiotic to destroy bacterial pathogens. This department grows cultures from major body systems such as the throat, urine, stool, wound, blood, eyes, ears, body fluids, nasal, abscesses, vagina, urethra, and tissues.

4. Specimen Collection:

The specimen collection department collects tissue, blood and urine samples from patients.

5. Urinalysis:

The urinalysis department performs chemical tests on urine specimens. Urine is tested for color, clarity, specific gravity, glucose, protein, ketones, occult blood, and pH. These tests are used to monitor urinary tract infections (UTI), metabolic diseases such as diabetes.

6. Blood Bank:

The blood bank or immunohematology department tests red blood cells from donors for antigens and serum from recipients for antibodies. Testing ensures that people receive compatible units of blood during a transfusion.

7. Immunology and Serology:

When invaded by microorganisms or other foreign bodies, the human body produces antibodies to protect itself from the threat. The immunology and serology department tests blood for antibodies (produced against pathogenic microorganisms) or antigens that may be indicative of many types of infectious disease. Routine tests include Widal test, C-reactive protein (CRP), rheumatoid factor (RF).

8. Genetics and Molecular Diagnosis:

Perform testing on DNA, RNA or chromosomes to help in detect microorganisms, look for genetic mutations and perform paternity tests, using techniques such as polymerase chain reaction (PCR), recombinant DNA technology and microarray.

9. Histopathology:

Used to detection tumor and cancer in cell tissue after surgery (by take a biopsy for microscopic examination).

10. Andrology:

Which include unit for seminal fluid analysis is a test of a man's sperm and semen. Also known as a sperm count or male fertility test, its results show how many sperm are released, aswell as how they're shaped and how well they move.

Laboratory Glassware:

Glassware are used in lab to contain or deliver liquids, usually manufactured from boro- silicate glass (Pyrex), is material with the following defined characteristics: resistant to the action of chemical and made to withstand sudden change of temperature. Such as cylinders, flasks, beakers, pipettes are graduated to certain volume, test tubes and funnels.

Sterilization and Disinfection:

Introduction:

Sterilization and disinfection are essential for ensuring that medical and surgical instruments do not transfer infectious pathogens to patients.

Define terms:

1. **Sterilization:** is a process that destroys or removes all forms of microbial life and is carried out in health-care means by physical or chemical methods.
2. **Disinfection:** is a process that removes many or all pathogenic microorganisms, except bacterial spores, on nonliving objects.
3. **Cleaning:** is a removal of visible soils (e.g., organic and inorganic material) from objects and surfaces. It is normally accomplished manually or mechanically using water with detergents or enzymatic products.
4. **Decontamination:** is removes pathogenic microorganisms from objects; so they are safe to handle, use or discard.
5. **Antisepsis:** is the use of chemicals (antiseptics) to make skin or mucus membranes free from pathogenic microorganisms.

Methods of Sterilization:

There are two basic methods of sterilization:

1- Physical Methods.

2- Chemical Methods.

1. Physical Methods:

a- Heat:

Heat is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell components. There are two types of heat sterilization:

1. Dry Heat Sterilization:

It is uses higher temperatures in the range of 160-180 °C and requires exposures time up to 2 hours, depending upon the temperature used. It is appropriate for sterilizing glassware and metal surgical instruments. Examples of dry heat sterilization are:

- Incineration (burning).
- Red heat.
- Flaming.
- Hot air oven: in this method; objects to be sterilized are exposed to high temperature (160°C) for period of one hour in an oven. **Advantages:** it is an effective method of sterilization of heat stable objects. The objects remain dry after sterilization. This is the only method of sterilizing oils and powders. **Disadvantages:** takes longer time.

2. **Moist Heat Sterilization:** it is involves the use of steam in the range of 121-134 °C. Steam under pressure is used to generate high temperature needed for sterilization. Moist heat may be used in three forms to achieve microbial inactivation:

- Pasteurization (at temperature below 100 °C):

This process was originally used by Louis Pasteur. This procedure is used in food and dairy industry. There are two methods of pasteurization, the holder method (heated at 63 °C for 30 minutes) and flash method (heated at 72 °C for 15 seconds) followed by quickly cooling to 13°C.

- Boiling (at temperature 100 °C):

Boiling water (100 °C) kills most vegetative bacteria and viruses immediately.

- Autoclaving (at temperature above 100 °C):

It is use pressurized steam to destroy microorganisms and is the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media and reagents. The conditions of sterilization in an autoclave are 15 minutes, 15 psi at 121°C.

b- Filtration:

It is a process does not destroys but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air.

c- Radiation:

Two types of radiation are used, ionizing and non-ionizing. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high energy rays with good penetrative power. Since radiation does not generate heat, it is termed "cold sterilization".

- **Non-ionizing rays:** are rays of wavelength longer than the visible light. Microbicidal wavelength of UV rays are in the range of 200-280 nm, with 260 nm being most effective.
- **Ionizing rays:** are of two types, particulate and electromagnetic rays. Electron beams are particulate in nature while gamma rays are electromagnetic in nature.

2 .Chemical Methods:

Sterilants are chemicals that destroy pathogenic bacteria from nonliving surfaces. Some chemicals have a very narrow spectrum of activity and some have a very wide one. Those chemicals that can sterilize are called chemosterilants. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics. An ideal antiseptic or disinfectant should have the following properties:

- Should have wide spectrum of activity.
- Should be able to destroy microbes within practical period of time.
- Should be non-toxic, non-allergenic, non-irritative or non-corrosive.
- Should not have bad odour.
- Should not be expensive and must be available easily.

Classification of Disinfectants:

1- Based on consistency:

- Liquid (e.g., alcohols, phenols).
- Gaseous (e.g. formaldehyde vapor, ethylene oxide)

2- Based on mechanism of action:

- Action on membrane (e.g., alcohol, detergent).
- Denaturation of cellular proteins (e.g., phenol).
- Oxidation of enzymes (e.g., H_2O_2, halogens).
- Damage to nucleic acids (e.g., formaldehyde).

Some Disinfectants with Their Applications:

1. Alcohols:

Mode of action: alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.
Examples: ethyl alcohol, isopropyl alcohol.
Application: 70% aqueous solution is more effective at killing microbes than absolute alcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin.
Disadvantages: skin irritant, volatile (evaporates rapidly), inflammable.

2. Aldehydes:

Mode of action: acts through alkylation of amino-, carboxyl- or hydroxyl group and probably damages nucleic acids.

Examples: formaldehyde.

Application: 40% formaldehyde (formalin) is used for surface disinfection and evaporation of rooms. Evaporation is achieved by boiling formalin.

Disadvantages: vapors are irritating (must be neutralized by ammonia), has poor penetration, leaves non-volatile residue.

3. Phenols:

Mode of action: act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

Examples: 5% phenol, chloroxylenol (Dettol).

Application: Joseph Lister used it to prevent infection of surgical wounds. They act as disinfectants at high concentration and as antiseptics at low concentrations. They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses.

Disadvantages: it is toxic, corrosive and skin irritant.

4. Surface Active Agents:

Mode of actions: they are disrupt membrane resulting in leakage of cell components.

Examples: these are soaps or detergents. Detergents can be anionic or cationic. The anionic detergents include soaps. Cetrimide act as cationic detergents.

Application: they are active against vegetative cells, mycobacteria and enveloped viruses. They are widely used as disinfectants at dilution of 1-2%.

الأسبوع الثاني

الهدف التعليمي: التعرف على التقنيات المستخدمة في تشخيص البكتريا والدم.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: The Basic Techniques that used in the Diagnosis of Blood and Bacteria:

1- The Diagnosis of Blood:

It is a specialized connective tissue consisting of cellular elements suspended in a pale-yellow fluid called the plasma.

- Blood circulates throughout the body, supporting the function of all other body tissues.
- Blood is both a tissue and a fluid. It is a tissue because it is a collection of similar specialized cells that serve particular functions. These cells are suspended in a liquid matrix (plasma), which makes the blood a fluid.

Composition of the Blood:

1. Formed elements:
- RBCs (red blood cells, 99.9% of formed elements).
- WBCs (white blood cells).
- Platelets.

2. Plasma.
- Water.
- Plasma proteins.
- Other solutes.

The Basic Techniques that used in the Diagnosis of Blood:

a- Hemoglobin (Hb):

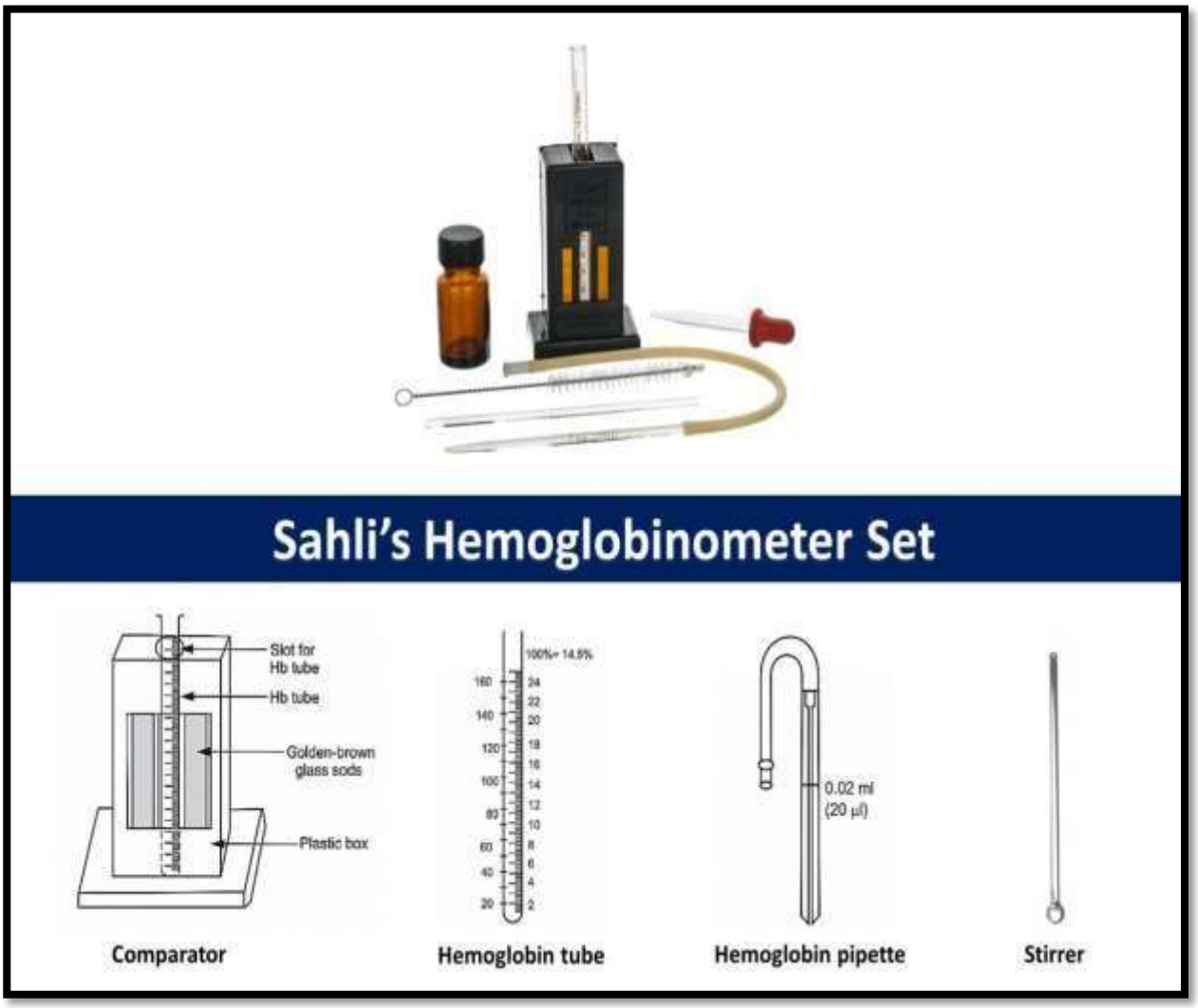
It is oxygen-carrying pigment in the red blood cells. Its molecular weight is 64,450 Da. Hemoglobin is a globular protein which is made up of four subunits. Each subunit

contains heme (an iron-containing porphyrin). Each heme molecule is conjugated to a polypeptide which is called the globin.

In each hemoglobin molecule there are four chains of polypeptides (two pairs) in hemoglobin A, which is normal adult human hemoglobin, the two polypeptides are called α chains and the other two, β chains. Each α chain consist of 141 amino acids and each β chain consist of 146 amino acids so there are 574 amino acids in whole molecule.

Sahli's or Acid Hematin Method:

Blood (from fingertip) is mixed with 0.1 N HCl resulting in the conversion of Hb to acid hematin which is brown in color. The solution is diluted till it's color matches with the brown colored glass of the comparator box. The concentration of Hb is read directly.



Normal Values of Hb Concentrations:

- Males: 15 ± 2 gm/dl.
- Females: 13.5 ± 1.5 gm/dl.
- Children: 10.5-13.5 gm/dl.
- Infant: 14.5-19.5 gm/dl.

b- Packed Cell Volume (PCV) or Hematocrit Test (HCT):

Packed cell volume or hematocrit test (PCV/HCT) is the volume of red blood cells after blood is centrifuged at a high speed.

Microhematocrit Method(Capillary Method):

Equipment Required:

- Capillary tubes 75 mm in length with an internal diameter of 1mm.
- Microhematocrit centrifuge.
- Reading device.

Procedure:

1- Fill the capillary tube with blood sample from fingertip. Leave the last 15 mm unfilled.

2- Cover the tube with clay. Make sure there is no air trapped between the clay and the column of blood.

3- Place the tube in the microhematocrit centrifuge with the covered end to the outer edge and centrifuge at 10000 rpm for 5 min.

4- Read the result using a microhematocrit reading device.

Normal Values of PCV or HCT:

- Males: 45±5%.
- Females: 41±5%**.**
- Children: 30-38% .
- Infant: 42-52%.

c- Erythrocyte Sedimentation Rate (ESR):

It is the measurement of the sedimentation of red blood cells when blood is allowed to settled in an open ended glass pipette fixed vertically on a stand for 1hour.

Anticoagulated whole blood in a pipette will be separated into an upper plasma layer and lower cell layer as a result of gravity interacting with the denser cells. The distance that cells fall within a specified time interval is the ESR. The ratio of fall, usually expressed as mm/hr.

Westergren's Method:

Equipment Required:

- Westergren pipette.
- Stainless steel rack for holding pipette.
- Timer.

Procedure:

1- Draw the venous blood sample (collected in EDTA tube) in the Westergren pipette to the 0 mm mark with a rubber teat.

2- Place the pipette exactly vertical in the rack which has a rubber cork at them bottom. Fix with adjustable screws after removing the teat.

3- Leave undisturbed for exactly 60 min free from vibrations and not exposed to direct sunlight.

4- Read the column of clear plasma above the column of sedimented red blood cells to the nearest mm.

Normal Values of ESR:

- Males: 0-10 mm1st hour.
- Females: 10-20 mm 1st hour.

d- Complete Blood Count (CBC):

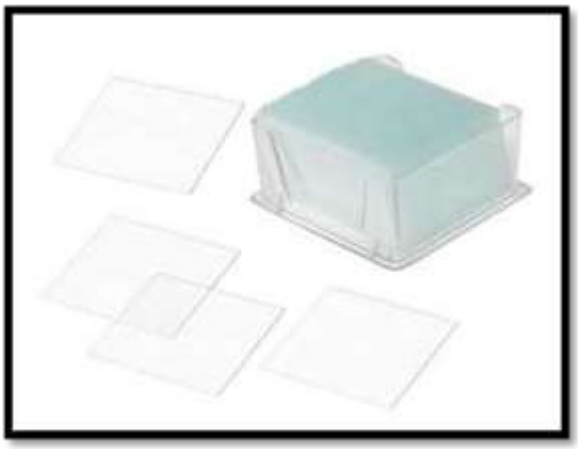
It is a measurement of the blood cells in a specific volume of blood.

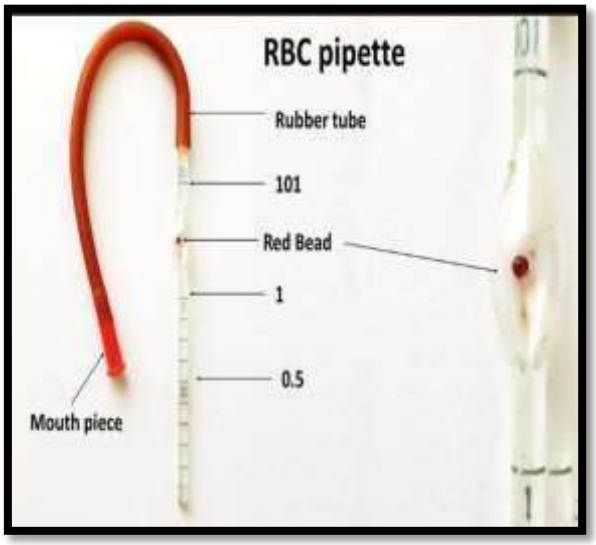
Procedure:

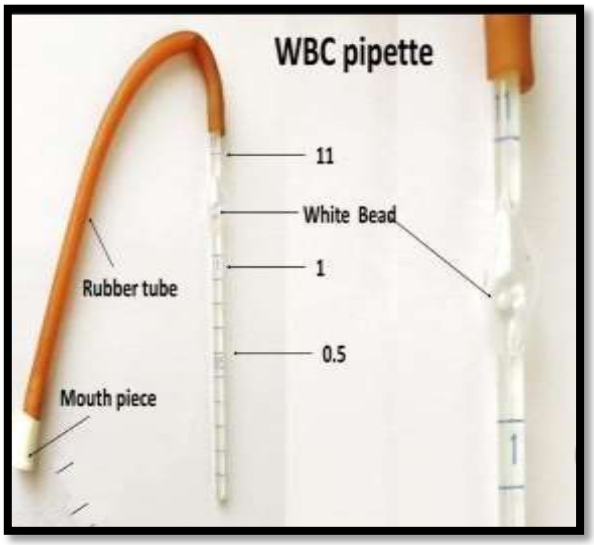
Blood cell count performed manually using a counting chamber (hemocytometer). Blood diluted with an appropriate solution, the diluted blood placed in the chamber under a cover slip and the cells in a ruled area were counted .

Equipment Required:

Hemocytometer: is an instrument used to count the blood cells. It includes: Neubauer's slide, cover slip, RBC pipette, WBC pipette**.**







- Red Blood Cells Count:

Procedure:

Blood is filled till mark 0.5 of the RBC pipette, and Hayem's fluid is then filled till mark 101. Both are thoroughly mixed and then few drops are discarded which contain just the diluting fluid in the stem. Thus, 1 portion out of 101 is discarded. So, 0.5 part of blood is in 100 parts of fluid or, 1 part of blood is mixed in 200 parts of fluid Thus, dilution factor for RBC counting is 200.

Normal Values of RBCs:

- Males: 4.8-5.5 million/mm^3.
- Females: 4.5-5 million/mm^3.

- White Blood Cells Count:

When blood is diluted with a weak acid solution; the RBCs are lyses while WBCs and platelets remain intact.

Procedure:

For WBCs counting; 0.5 part of blood is mixed in 10 parts of fluid solution, 1 part of blood is in 20 parts of fluid and thus, dilution factor for WBC counting is 20.

Normal Values of WBCs:

- Adult: 4000-11000/mm^3.

- Children: 5000-15000/mm^3.

- Platelets Count:

Procedure:

Whole blood is diluted with a 1% ammonium oxalate solution, all erythrocytes are lysed while the leukocytes, platelets, and reticulocytes remain intact. The standard dilution for platelets count is 1:100. The dilution is mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution is mounted on a hemocytometer.

Normal Value of PLs:

150000-400000/mm^3.

d- Differential Count (DC):

It is a process for enumerate different types of WBCs.

- Slide Method:

Procedure:

1- Put a drop of blood on the surface of a slide, 1 cm far from the edge.

2- Use a edge slide (spreader) in an angle of 45° from the primary slide to spread the drop of blood.

3- After dryness of the smear; slide is stained with Leishman's stain.

4- Examine the slide under the high dry objective to observe cell distribution, red cells morphology and to screen for abnormal cells that might be missed during the count.

5- A drop of oil added and use oil-immersion lens, the count should be made in the feathered edge area of the smear, where the RBCs are evenly dispersed with little overlap .

Normal Values of Different Types of WBCs:

- Neutrophil: 45-75% .
- Lymphocyte: 25-45% .
- Monocyte: 2-8% .
- Eosinophil: 2-6% .
- Basophile: 0-1% .

2- The Diagnosis of Bacteria:

Collection of Samples for Culture:

a- General information required:

- Patient’s full name and date.
- Source of sample or collection site.
- Sample type and test desired.

b- Method of obtain sample correctly:

- Use a sterile container.
- Label correctly and send the sample to the laboratory promptly.
- Avoid contamination of the container.

c- Timing of collection:

Sputum, urine, stool, etc. are best collected in early morning and sent to the laboratory the same day.

Blood Sample:

- A blood culture requires two bottles of blood; one for aerobic and one for anaerobic culture. Each blood culture should be collected from a separate venipuncture.
- Collect blood sample before treatment is initiated, if possible.

Upper Respiratory Tract Sample:

- Nasopharyngeal culture is obtained by inserting a thin sterile swab gently through the nose to touch the pharynx; gently rotate and remove.
- Throat culture is obtained by introducing a sterile swab into the mouth. Use a tongue blade to avoid contaminating the sample with oral secretions. Firmly swab both tonsillar fossae, posterior pharynx, and any inflamed or ulcerated areas.

Lower Respiratory Tract Sputum Sample:

- Rinsing the mouth with saline or water (but not mouthwash) may reduce contamination with normal oropharyngeal flora.
- Encourage deep cough with expectoration of the sputum into a sterile sample collection cup that is labeled with the patient's name and second patient identifier.
- Do not send saliva (spit) for culture.
- When the patient is unable to cough; an alternative method may be ordered, such as: induced sputum, tracheal aspiration, bronchial washings.

Wound Exudate Sample:

- Gently cleans the area, using dry sterile gauze to remove any contaminants.

- Using a sterile red-stopper swab, introduce deeply enough to obtain a moist sample; release the swab in the container. Do not break the container.

- Store at room temperature.

Stool Sample:

- A small amount is all that is required, about the size of a walnut. If several different types of cultures are requested, a walnut-sized sample for each one. Place the sample in transport medium or in a sterile leakproof container.
- When stool cultures are not readily obtainable, rectal swabs are acceptable; however, it must be indicated whether the sample is a stool or a rectal swab.

Bacteriological Examination of Samples:

A general plan for examination samples is as follows:

1-Macroscopic Examination: note the following

1. Color, opacity, consistency.
2. Presence of blood, mucus or pus.
3. Presence of macroscopic bodies, such as parasites.

2-Microscopic Examination:

a- Unstained film or wet preparation: when looking for cells or casts in urine deposit and protozoa parasites in stool or looking for motile bacteria.

b- Stained film by:

- Simple stain.

- Gram stain.

- Acid-fast bacilli stain.

- Special stain.

- Negative staining.

Microscopic Appearance of Bacteria (Wet and Stained Smears):

Note shape, size, arrangement, motility, staining reaction, spores, capsules, pleomorphism andGram stain reaction.

3-Culture: inoculated accurate media according the suggested microorganisms found in the sample, then incubated aerobically and anaerobically, mostly using blood agar, MacConkey agar and special media.

4- Examination of Cultures:

a- Keep extensive notes on the examination of the cultures set up as follows:

Solid Cultures:

Note the shape, color, size, consistency, hemolysis.

Liquid Cultures:

Note nature of the medium such as color, turbidity, deposit.

b- Biochemical Tests: oxidase, catalase, coagulase, IMVIC tests, motility**.**

c- Serology Tests and Serotyping: agglutination test with specific anti sera.

d- Antimicrobial Susceptibility Testing.

5- The Report:

For the final report, however, it is only necessary to report the organism or organisms seen insmear and isolated on culture together with the sensitivity pattern.

الأسبوع الثالث

الهدف التعليمي: التعرف على السلامة المختبرية وأنواع المخاطر في المختبر.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Laboratory Safety:**

The Occupational Exposure to Hazardous Chemicals in Laboratories Standard:

The provisions in the occupational Exposure to hazardous chemicals in laboratories standard cover the routes of exposure, chemical inventory, storage of chemicals and chemical spills.

1. Routes of Exposure:

There are several ways that a hazardous chemical can enter the body. Hazardous chemicals can enter through the mouth or a cut on the hand, but also through the lungs or eyes. Some hazardous chemicals can enter the body through intact skin.

2. Chemical Inventory:

Laboratories keep an inventory of all chemicals used for testing and other procedures. A chemical inventory is useful in other ways. For example, laboratories share their chemical inventory with the local fire department, so that fire department personnel can come prepared to resolve a hazardous chemical spill, an explosion, or another type of chemical emergency.

3. Storage of Chemicals:

Storage of chemicals is important to ensure not only the safety of the individuals working in a laboratory but also the safety of others in the same building.

Rules for Store Chemicals:

• Store similar chemicals together to minimize interactions between chemicals.

• Keep flammable chemicals together in a flammable-storage cabinet.

• Store hazardous chemicals separately from nonhazardous chemicals.

• Check for improperly stored chemicals, leaking containers.

4. Chemical Spills:

Although most individuals are extremely careful when handling chemicals, chemical spills are inevitable. The person spilling the chemical should take responsibility for cleaning it up. Notify the supervisor and report the spilled chemical and location.

The Bloodborne Pathogens Standard:

The bloodborne pathogens standard was issued in 1991 and most recently updated in 2012. Its purpose is to protect workers from microbiological pathogens that are carried in blood and body fluids.

Requirements of the Bloodborne Pathogens Standard:

1. Train workers before they are exposed to blood and body fluids.

2. Offer each employee the hepatitis B vaccination series.

3. Provide appropriate PPE (personal protective equipment) such as gloves, laboratory coats,face shields, and goggles, and instruct personnel on when and how to use this equipment.

Clinical Laboratories and Biosafety Levels:

Clinical laboratories represent a unique working environment because not only hazardous chemicals but also biological hazards are part of the environment. Biosafety levels are used to describe the potential biological hazard and the function of the laboratory. There are four biosafety levels, with 1 being the safest environment and 4 being the most hazardous environment.

1. Biosafety Level 1:

A biosafety level 1 laboratory contains equipment, practices, and facilities that will be used with organisms that do not consistently cause disease in healthy adults.

2. Biosafety Level 2:

A biosafety level 2 laboratory contains equipment, practices, and facilities used to identify and characterize moderate- risk agents derived from the community that cause disease in immunocompromised and immunocompetent people.

3. Biosafety Level 3:

A biosafety level 3 laboratory contains equipment, practices, and facilities used to identify and characterize organisms that cause severe or potentially lethal infections transmitted through inhalation.

4. Biosafety Level 4:

A biosafety level 4 laboratory contains equipment, practices, and facilities used to identify and characterize extremely hazardous infectious organisms, usually transmitted through aerosols, that frequently cause fatal diseases, diseases with no cure or treatment, or diseases with an unknown transmission mechanism.

Laboratory Safety Equipment:

1. Biological safety cabinet.
2. Fume hood.
3. Needle stick prevention engineering control.
4. Fire suppression systems.
5. Pipetting aids.
6. Eye wash stations.
7. Emergency showers.

الأسبوع الرابع

الهدف التعليمي: التعرف على العينات وأنواعها وطرق جمعها وكيفية التعامل معها.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Sample Collection:**

Collection procedures:

Collection procedures will vary according to sample type and the required analyses.

1- Blood Collection:

Blood can be collected from three different sources:

a- Venous blood. b- Arterial blood. c- Capillary blood.

a- Venous Blood: it is the most commonly required blood sample. The majority of routine tests are performed on it. This is obtained directly from the vein. Best site for the collection of venous blood is the deep veins of the antecubital fossa.

Select Venipuncture Site:

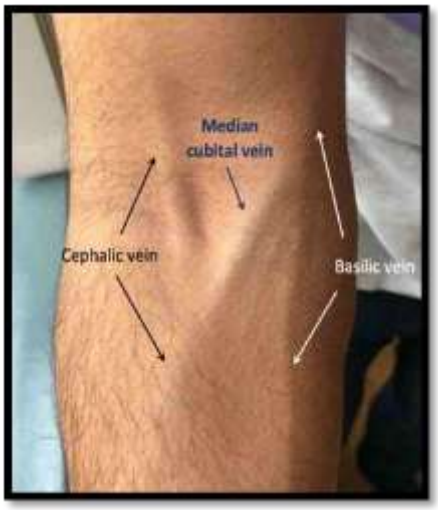
In most cases, blood will be drawn from an arm vein, since these vessels are usually large, close to the skin surface, and easy to penetrate. An alternate sites must be selected because of burns, amputation, or other medical consideration. Alternate sites include the back of the hand,ankle, or foot.

Materials:

- Tourniquet.
- Disposable sterile needle.
- Cotton swap.
- Alcohol.

Procedure:

- If multiple samples are to be collected, it is better to use a butterfly needle.
- Palpate the vein.
- After all the precautions have been made and the patient is comfortable, a tourniquet should be applied on the upper arm.
- The area to be punctured should be sterilized with a spirit swab and allowed to dry for awhile.
- Insert the needle into the vein in such a way that it traverses a little distance under the skin.
- Do not enter the vein directly and vertically, as chances of puncturing the other side of thevenous wall.
- Now draw the blood according to your requirement.
- After this, first remove the tourniquet and place a clean spirit swab at the site of vein-puncture and with draw the needle.
- Put the blood into a suitable tube, already labeled.



b- Arterial blood: it is used for the estimation of blood gases. This sample is occasionally required.

c- Capillary blood: it is a great value in children and adult with difficult veins. Select a suitable site for puncture, the ball of the finger or the side of the thumb. Form a baby, it is bestto obtained blood from the base of the heel, only few tests can be performed.

Materials:

- Cotton swab. - Alcohol. - Disposable lancet. - Capillary tube.

Procedure:

- Rub the area by massaging.
- Sterile the area with 70% alcohol by the cotton swab and allow to dry.
- Punch the area by disposable lancet, the puncture should be about 3 mm.
- Wipe off the first drop of blood and a little pressure is applied.
- Never press on blood.
- Take the blood by capillary tube .
- Apply slight pressure over the area.
- Sterile the area with 70% alcohol by the cotton swab.

2- Urine collection:

Many analytes, such as steroids, hormones, and a wide variety of drugs and their metabolites, can be measured in urine, making it a appropriate sample for a variety of tests. Urine collection can performed under several conditions, depending on the required test:

a- First morning samples: they are collected in the cup immediately upon rising in the morning, recommended for analytes requiring concentration for detection in laboratory assays.

b- Random urine samples: they are appropriate for drug monitoring and cytology tests.

c- Fractional samples: the patient fasts after the last evening meal, and the urine is collected in the cup. These samples are used to compare urine analyte levels with their concentrations inblood.

Labeling of Samples:

It is very important to label the following on the sample and request from patient:

- Name of patient.
- Ward which patient in it (number).
- The bed that patient sleep on it (number).
- Date.
- Age.

Sample Transport:

It is refers to the process of moving biological or chemical samples from their collection point to a laboratory for analysis, ensuring the sample's integrity throughout the process. This involves careful handling , packaging, and adherence to regulations to prevent contamination or degradation, ultimately guaranteeing accurate test results

Key Aspects of Sample Transport:

1- Maintaining Sample Integrity: the primary goal is to preserve the sample's original state, preventing any changes that could affect test outcomes.

2- Proper Packaging: this often involves triple packaging , including a leak-proof primary container, a durable secondary container, and a sturdy outer packaging.

3- Temperature Control: many samples , like blood or serum , require specific temperature conditions (e.g., refrigeration or freezing) during transport.

4- Adherence to Regulations: transport often needs to comply with regulations from various bodies, including national transport agencies, IATA, and others, to address potential hazards.

5- Minimizing Hazards: procedures are in place to minimize the risk of infection or contamination during transport, particularly for potentially infectious samples.

6- Specialized Systems: some laboratories use automated systems like pneumatic tube systems or rail-based transport for efficient and safe movement of samples.

Sample Preparation:

It is a crucial step in any analytical method. It involves transforming a sample into a state that is suitable for analysis , ensuring the sample accurately represents the material being studied , and minimizing potential interferences. This process can include a variety of techniques like extraction, filtration, dilution, and chemical treatment, depending on the specific analysis and sample type.

Key aspects of sample preparation:

1- Extraction: isolating the analyte of interest from the sample matrix.

2- Purification: removing interfering substances that can hinder analysis.

3- Concentration: adjusting the analyte concentration to be within the detection range of the analytical instrument.

4- Homogenization: ensuring the sample is uniform, especially for solid samples.

5- Interference removal: eliminating substances that can interfere with the analysis.

الأسبوع الخامس

الهدف التعليمي: التعرف على أنواع الأوساط الزرعية وطرق زرع الأحياء المجهرية.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Culture Media:**

Culture media are specific mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi (yeasts and molds).

Uses: isolate, identify and study the characteristics of microorganism.

Bacterial Culture Media:

Basic Requirements for Bacterial Culture Media:

1- Energy source.

2- Nutrition which includes:

a- Carbon source e.g.: sugars, carbohydrate, CO_2.

b- Nitrogen source e.g.: peptone.

c- Others e.g.: sulfur , phosphorous , metal salts , trace elements , vitamins , essential metabolites.

3- pH (acid, alkaline, neutral).

4- O_2 (aerobic or anaerobic).

5- Time of incubation.

6- Moisture.

7- Temperature: the optimum temperature of pathogenic bacteria is 37° C (human body temperature).

Classification of Culture Media:

Bacterial culture media can be classified based on consistency, nutritional component and its functional use or application.

1. Based on Consistency:

Liquid Media:

In liquid media (without agar), bacteria grow producing turbidity, surface pellicle , granular deposits. Culturing bacteria in liquid media has some problems: properties of bacteria are not visible in liquid media and presence of more than one type of bacteria cannot be detected.

Solid Media:

Any liquid medium can be solid by the addition of certain solidifying agents such as agar (1.5-2%).

Agar: is the most commonly used solidifying agent. It is an unbranched polysaccharide obtained from the cell membranes of some species of red algae such as the genera Gelidium. It is composed of two long-chain polysaccharides (70% agarose and 30% agaropectin). It is melts at 95 ^{o}C and solidifies at 42 ^{o}C, doesn't contribute any nutritive property. It is not hydrolyzed by most bacteria and it is usually free from growth promoting or growth slow down substances.

Semi-solid Media:

Reducing the amount of agar to 0.2-0.5% to obtain a semi-solid media. Such media are soft and are useful in bacterial motility test. Certain transport media such as Stuart's and Amies media are semi-solid in consistency.

Biphasic Media:

Sometimes, a culture system consist of both liquid and solid media in the same bottle. This is known as biphasic media. The inoculum is added to the liquid medium and when subcultures are to be made, the bottle is simply slanted to allow the liquid to flow over the solid medium.

2. Classification Based on Nutritional Component:

Simple Media: such as peptone water and nutrient agar which can support most non-fastidious bacteria.

Complex Media: such as blood agar have ingredients whose accurate components are difficult to estimate.

Synthetic or Defined Media: such as Davis and Mingioli media are specially prepared mediafor research purposes where the composition of every component is well known.

Note: the bacteria that are able to grow with minimal requirements are said to **non-fastidious** and those that require extra nutrients are said to be **fastidious**.

3. Classification Based on Functional Use or Application:

Basal Media: are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal media.

Enriched Media: are used to grow nutritionally fastidious bacteria. Addition of extra nutrients in the form of blood, serum, egg yolk etc., to basal medium makes them enriched media. Blood agar, chocolate agar, Loeffler's serum slope etc. are few of the enriched media.

Blood Agar: is prepared by adding 5-10% blood (by volume) to a basal medium such as nutrient agar or other blood agar bases. After the blood agar base is autoclaved, blood is addedto the medium at temperature just above the solidifying point of agar. The mixture is then poured in the plates and allowed to solidify. Blood agar is useful in detection hemolytic properties of certain bacteria.

Selective Media: are designed to inhibit unwanted commensal or contaminating bacteria and help to select pathogen from a mixture of bacteria. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these. MacConkey's agar used for enterobacteriaceae members contains bile salt that inhibits most gram-positive bacteria.

Differential Media: distinguish one microorganism type from another growing on the same medium. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red or methyleneblue) added to the medium to visibly indicate the defining characteristics of a microorganism e.g.: MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

When a particular substrate (carbohydrate) is inserted into a medium and a mixture of bacteria inoculated on it, only that bacterium that can ferment it produces acid. This change in pH is detected by using a pH indicator inserted in the medium and the bacterium that can ferment the sugar appears in a different color. This approach is used in MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

MacConkey's agar is the most commonly medium used to culture and identify gram negative bacilli (especially enterobacteriaceae members). It contains bile salts (selective agent), lactose (sugar), peptone and neutral red (pH indicator), agar and water. Those bacteria that can ferment lactose produce pink colored colonies where non-lactose fermenting colonies produce colorless colonies.

Transport Media: such media prevent drying of sample, keep the viability of all organisms inthe sample without altering their concentration. Some of these media (Stuart's and Amie's) aresemi-solid in consistency.

Anaerobic Media: anaerobic bacteria need reduced oxidation-reduction potential and extra nutrients. Such media may be reduced by physical or chemical means. Boiling the medium used to eject any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycolate, 0.1% ascorbic acid, 0.05% cysteine or red-hot iron filings to obtain a anaerobic medium. Robertson cooked meat that is commonly used to grow Clostridium spp. medium.

Samples Types:

A wide variety of samples types may be collected:

- Blood and blood fractions (plasma, serum, buffy coat, red blood cells).
- Urine.
- Feces.
- Semen.

- Saliva/buccal cells.
- Cerebrospinal fluid (CSF).
- Tissue (from surgery, autopsy, transplant).
- Placental tissue, meconium, cord blood.
- Bone marrow.
- Breast milk.
- Bronchoalveolar lavage.
- Cell lines.
- Exhaled air.
- Fluids from cytology (ascites, pleural fluid, synovial fluid, etc.).
- Hair.
- Nail clippings.

Bacterial Growth Curve:

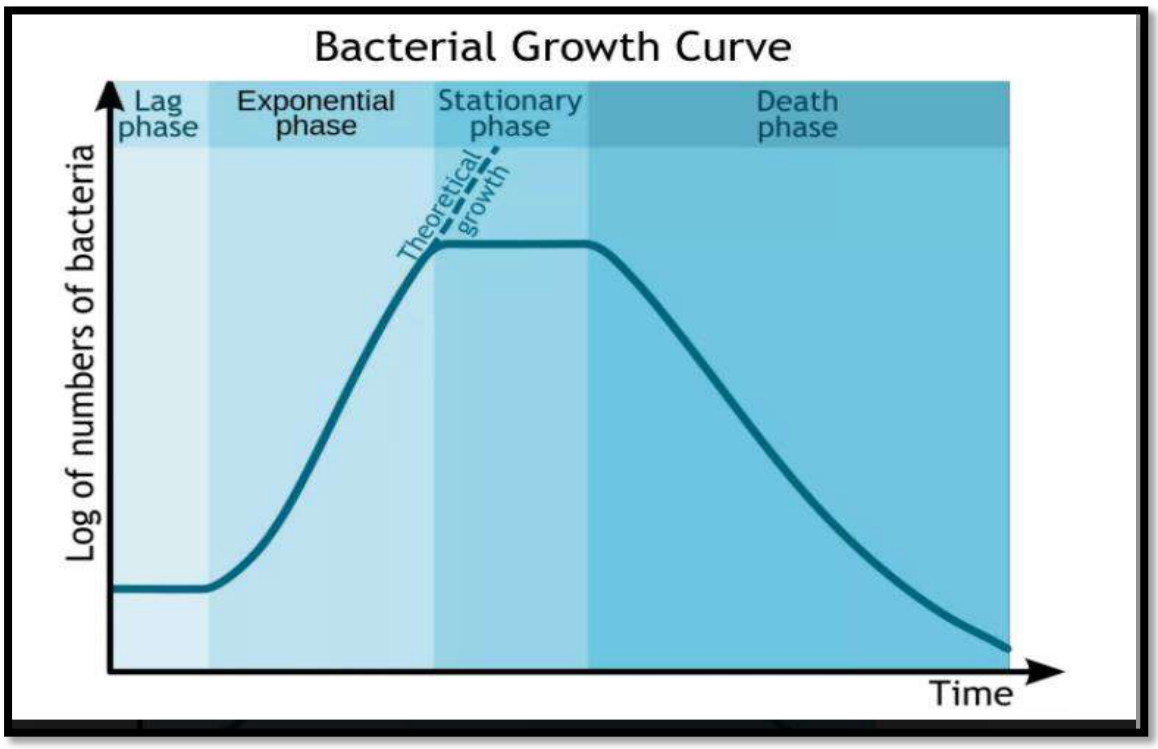
Bacterial growth curve refers to the increase in the number of bacterial cells in a population. Bacterial growth can be divided into several phases:

1. Lag Phase: In this initial phase, bacteria adapt to their environment, synthesize essential enzymes, and prepare for rapid growth. Cell numbers do not increase significantly during this phase.

2. Logarithmic (Exponential) Phase: During this phase, bacterial growth is characterized by exponential population growth. Bacteria divide rapidly and double in number with each generation as long as the environment remains favorable. This phase is ideal for studying bacterial physiology and for industrial applications like fermentation.

3. Stationary Phase: In the stationary phase, the growth rate of bacteria slows down, and the number of cells entering division equals the number of cells exiting division. This phase occurs when resources become limited or waste products accumulate.

4. Death Phase: In the death phase, the number of viable bacterial cells starts to decline. This phase is characterized by cell death and a decrease in the overall population.



Methods of Culturing Bacteria:

Inoculation: is transfer of a bacterial sample onto a growth media for the purpose of growingthe sample.

Aseptic Technique During Inoculation of Culture Media:

1- Decontaminate the workbench before and after the work of the day. 2- Use facemask and gloves during handling highly infectious samples.

3- Flame sterilizes wire loops, straight wires, and metal forceps before and after use.

4- Flame the neck of sample and culture bottles, and tubes after removing and before replacingcaps and plugs.

Methods of Inoculation:

1- Culturing on Solid Media: this is including the followings

a- Plate Cultured Methods:

These methods are designed for culture from broth, agar plate or slopes. After preparing the solid media in Petri-dish must be stored in the refrigerator until inoculation. The agar surface should be free of water of condensation before the streaking is done. The drying of the plates is performed by placing the surface of the lid onto an incubator shelf (at 37 ^{o}C).

Streaking Plate Method:

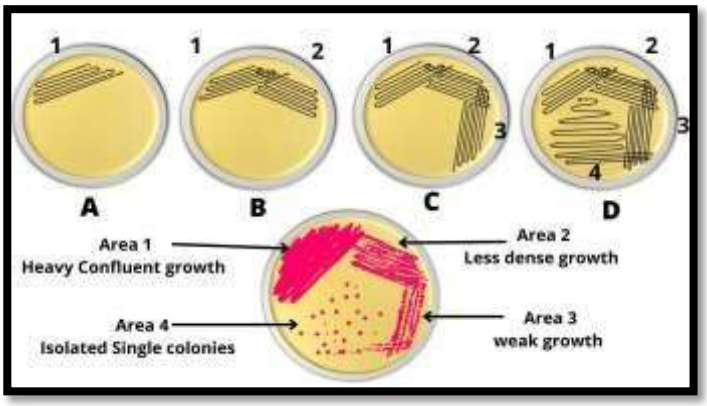
It used for obtaining single colonies (isolated colonies) from food and/or cultures, colonies canbe identified and further evaluated.

Procedure:

1- A small amount of sample is placed on the side of the agar plate (either with a swab, or as adrop from an inoculating loop).

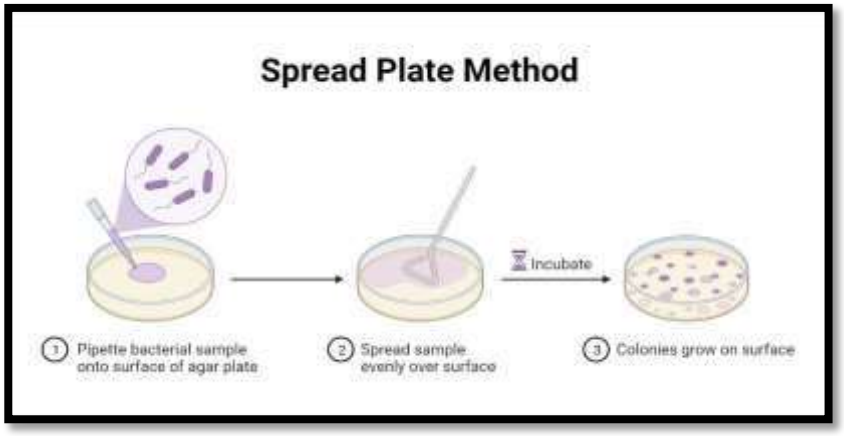
2- A sterile loop is then used to spread the bacteria out in one direction from the initial site of inoculation. This is done by moving the loop from side to side, passing through the initial site. 3- The loop is then sterilized (by flaming) again and the first streaks are then spread out themselves.

4- This is repeated 2-3 times, moving around the agar plate as shown in the figure.



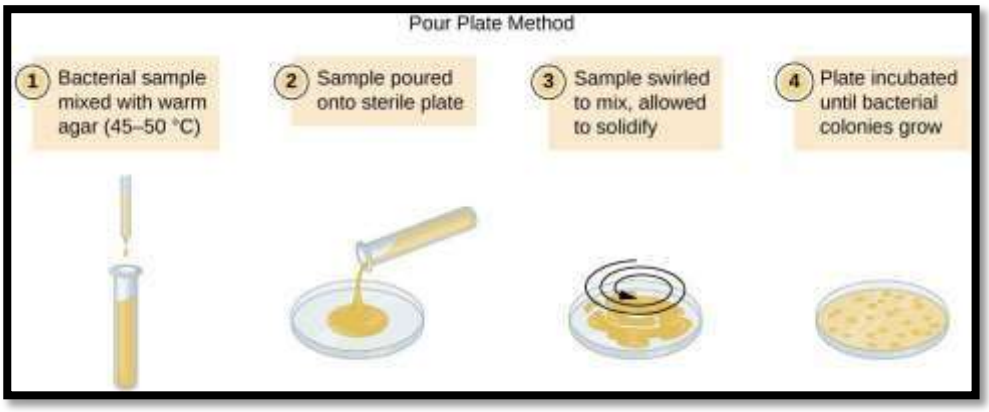
Carpet Plate Method:

This method is prepared by flooding the surface of plate with suspension of bacteria. It is provides uniform surface growth of bacteria; it is useful for bacteriophage typing and antibiotic sensitivity test and this method of streaking may be used either for culture from solid media or for heavy broth culture and smeared over the whole surface, using a sterile spreader or loop or swabs.



Pour Plate Method:

About 15-20 ml of agar media are melted and left to cool in water bath at 45-50 °C. Appropriate dilution of inoculum is added in 1 ml volume to molten agar and mixed well. Contents of tube are poured in Petri dish. They are allowed to set and after incubation colonieswill be seen distributed through out of the depth medium. The colonies growing in medium. This method gives viable bacteria count in suspension, it is recommended method for food microbiology.



a- Tube Culture Methods:

Slope (Slant) Culture Stock Method:

Many tests devised to differentiate organisms require solid cultures. It is not always necessary to grow an organism on a whole Petri dish of medium, and slope cultures often be enough. Slops or slants are tubes or bottles containing a small quantity of medium ,that has been allowed to solidify with the bottles slightly raised at one end . Such slopes are used only for maintenance or biochemical tests once the organism has been isolated in pure culture, they cultured by streaking the surface of slope media.

Deep Culture Method:

Anaerobic organisms require an oxygen-free atmosphere. For cultivation of these organisms shake or deep cultures are sometimes made. The medium is distributed in 150mm x 20mm tubes to a depth of 6-7cm and allowed to solidify. For use, the medium is melted, cooled at about 45 °C, inoculated with organism, and mixed by rotation between the palms of the hands.When it has solidified, the culture is incubated and the anaerobic

organisms grow at the bottom of the tube. These shakes or deep tubes can also be used for counts of viable organisms.

Roll Tube Method:

The roll tube method is also useful for counting viable organisms. The medium is distributed in tubes, 1-2 ml per tube, and stored. For use, the medium is melted, cooled to approximately 50° C, and a known dilution of the test sample is added. The tube is then titled and rolled between finger and thumb, allowing the medium to run all rounds the sides of the tube just below the half way mark. This rolling is carried out under cold tap water. A thin film of agar solidifies around the sides of the tube, which is inverted for incubation.

Stab Culture Method:

It is prepared by puncturing charged long straight wire (4.5-5 cm). Stab culture is employed mainly for demonstration of gelatin liquefaction and motility test and for maintaining stock culture.

Description of Colonies on Solid Cultures:

Colonies of bacteria are described as follows:

- Shape: circular, irregular, radiating or rhizoid.
- Surface: bacterial colonies are frequently shiny and smooth in appearance. Other surface descriptions might be: veined, rough, dull, wrinkled (or shriveled), glistening.
- Color: it is important to describe the color or pigment of the colony. Also include descriptive terms for any other relevant optical characteristics such as: opaque, cloudy, translucent, iridescent.
- Size: surface colonies are measured in millimeter; they are 2-3 mm in diameter. Smaller onesmay be less than (about 0.5-1 mm).
- Elevation: may be raised, low convex, implicated or dome.
- Edges: mostly edges are entire, sometimes crenate, fimbriated or effuse.
- Pigmentation: some organism may produce pigmented colonies (Staphylococcus , Pseudomonas).
- Opacity: colonies on nutrient agar may be transparent, translucent or opaque.

- Consistency: mostly soft and butyrous and may be hard, firm, mucoid, tenacious, dry, adherent to medium, friable and membranous.
- Contiguity: may be discrete or swarming.
- Changes in the medium: colonial growth may bring about color changes in the media themselves produce soluble pigment that diffuse in to the medium and some organism hemolysis the blood of medium around the colony.
- Emulsifiability: growth of some bacteria is easily emulsifable (e.g. *Escherichia coli*, Salmonella) whereas growth of *Neisseria catarrhalis* is not emulsifable and form granules.
- Odor.

2- Culturing on Liquid Media:

- Media prepared in tubes or bottles or flasks and inoculated by touching with a charged loop.
- Liquid culture is preferred when large and quick yield is required.
- The major disadvantage of liquid culture is that it does not provide pure culture from mixedinocula.
- This method constitutes a simple technique and used for liquid culture largely but it may alsobe used for culture from solid media.

Description of Growth in Liquid Cultures:

Growth in liquid medium is described as follows:

- Turbidity: clear or turbid.
- Deposit: growth of *Streptococcus pyogenes* is characterized by deposit at the bottom of the tube.
- Surface growth: is related to aerobic nature of organism.
- Color changes: some organisms produce water soluble pigment which after diffusion changethe color of medium e.g. *Pseudomonas pyocyaneus*.

الأسبوع السادس

الهدف التعليمي: التعرف على البول ومكوناته والفحص المجهري والفيزيائي والكيميائي لعينة البول.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Urine Sample:**

Urine: is a liquid that formed in the kidneys, it is a product of ultrafiltration of plasma by therenal glomeruli.

The Composition of Urine:

- Water (about 95% of urine).
- Urea.
- Creatinine.
- Uric acid.
- Electrolytes.

The Factors Affecting of the composition of Urine:

1- Diet and nutritional status.

2- Condition of body

metabolism.3- Ability of

kidney function.

4- Level of contamination with pathogenic microorganisms (bacteria) or even non-pathogenicmicroflora.

Collection of Urine Sample:

- Early morning sample.
- Random sample.

- 24 hours sample.

- Mid-stream sample.

A mid-stream urine sample means don't collect the first or last part of urine that comes out.

Preservation of Urine Sample:

Changes that affect the chemical or microscopic properties of urine occur if urine is preservedat room temperature for more than 1 hour.

a- Refrigeration: is a most common method for preserving urine, it is prevents bacterialgrowth for 24 hours.

b- 20 ml of 2N HCl can be added to container prior to the collection. Urine preserved in this method is suitable for estimation of urea, ammonia, and Ca^{++} . Chloroform, toluene, and thymol have also been used as preservative.

General Urine Examination (GUE):

1- Macroscopic Examination (Physical Examination and Chemical Examination).2- Microscopic Examination.

Physical Examination:

a- Color:

Normal----------pale to deep amber.

Milky----------chyluria, UTI (urinary tract inflammation).

Reddish----------bleeding in UTI.

Greenish-yellow, dark-brown, orange-yellow----------jaundice.

b- Odor:

Normal---------- aromatic.

Abnormal---------- non aromatic.

c- Specific gravity: density of a substance compared to the density of water.Normal---------- 1.005 to 1.030.

Increased ---------- dehydration, diabetes.

Decreased ---------- kidney disease, diuretic, increased fluid intake.

Chemical Examination:

a- pH: is a measure of acidity or alkalinity of

urine.Normal---------- 5.5 to 8.0.

Affected by:

- Diet.
- Medications.
- Kidney diseases.
- Starvation.

b- Protein:

Normal---------- absence.

Abnormal-------- presence (proteinuria).

b- Glucose:

Normal---------- absence.

Abnormal-------- presence (diabetes).

c- Ketones: end product of fat

metabolism.Normal absence.

Abnormal--------presence (diabetes, fasting, dieting, high fat diet).

d- Blood:

Normal----------absence.

Abnormal------ presence (injury, infection, menstruation, kidney diseases).

e- Bilirubin: break down product of hemoglobin.Normal absence.

Abnormal------ presence (liver diseases).

Microscopic Examination:

Procedure:

a- Centrifugation:

- Shake the urine sample to make the sample homogeneous.
- Put 10 ml of urine sample into test tube.
- Centrifuge the urine sample for 5 minutes at 2000 RPM and discard from the liquid part.

b- Examination:

- Place a drop of sediment on a glass slide and cover the drop with cover slide.
- Place the glass slide on the microscope stage.
- Examine for the elements: WBCs, RBCs, epithelial cells, yeast, bacteria, mucous, crystals, pus and casts.

الأسبوع السابع

الهدف التعليمي: التعرف على صفات عينة البراز والفحص العام لها.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Stool Sample**

General Stool Examination:

General Stool Examination (GSE) is carried out in laboratories for various diagnostic purposes. Examination of stool is very helpful in the diagnosis of disease of the gastrointestinal tract. Mostly a clean container which does not contain any detergent or disinfectant is sufficient for all types of stool examinations including stool culture. It consist the following tests:-

a. PHYSICAL EXAMINATION OF STOOL:

Sample should be examined immediately after collection. Samples left standing prolonged will deteriorate helminthes, Ovum, other parasites and increase the numbers of monilia and bacteria which gives wrong results, however the following aspects of stool should be examined:

1- Quantity: the adult person excretions about 150-250 gm. /day of feces, about (1/3- 1/2) of feces dry weight is bacteria.

2- Consistency and form: normal stool is well formed. But in constipation (Dehydration) the stool is solid (Hard) and the semi-solid (soft or loose) seen when taking certain medications and laxatives. In abnormal cases such as diarrhea and dysentery the stool appear liquid, or watery in nature. In cholera the stools have a rice water appearance. In cases of malabsorption of fats the stools are pale bulky and semi-solid.

3- Color:

- Normal colors of stools are light to dark brown due to the Presence of bile pigments.
- Dark black: in cases with bleeding into the intestinal tract the stools become dark tarry in nature due to the formation of acid hematin , if the bleeding is in the small intestines. In case

of bleeding in large intestines or rectum stool color may be bright red due to fresh blood.

- Red color: resulted from eating certain colorful foods such as red beets.
- Clay color: the stool may be clay colored due to absence of stercobilinogen in biliary tract obstruction.

4- Odor: the normal fecal odor of stool resulted from indole and skatol. Odor of stools may become offensive in conditions like, Intestinal amoebiasis. In cases of bacillary dysentery and cholera the stools are not foul smelling due to the absence of fecal matter.

5- Blood:

1- The blood is present on the outer surface of the feces and this caused either by contamination from menstrual cycle blood in women or bleeding hemorrhoids from the blood vessels.

2- Blood should be noted in stools if present as it is indicative of Ulceration or presence of any other pathology like malignancy.

6- Mucus: Is present in certain conditions like amoebic or bacillary dysentery.

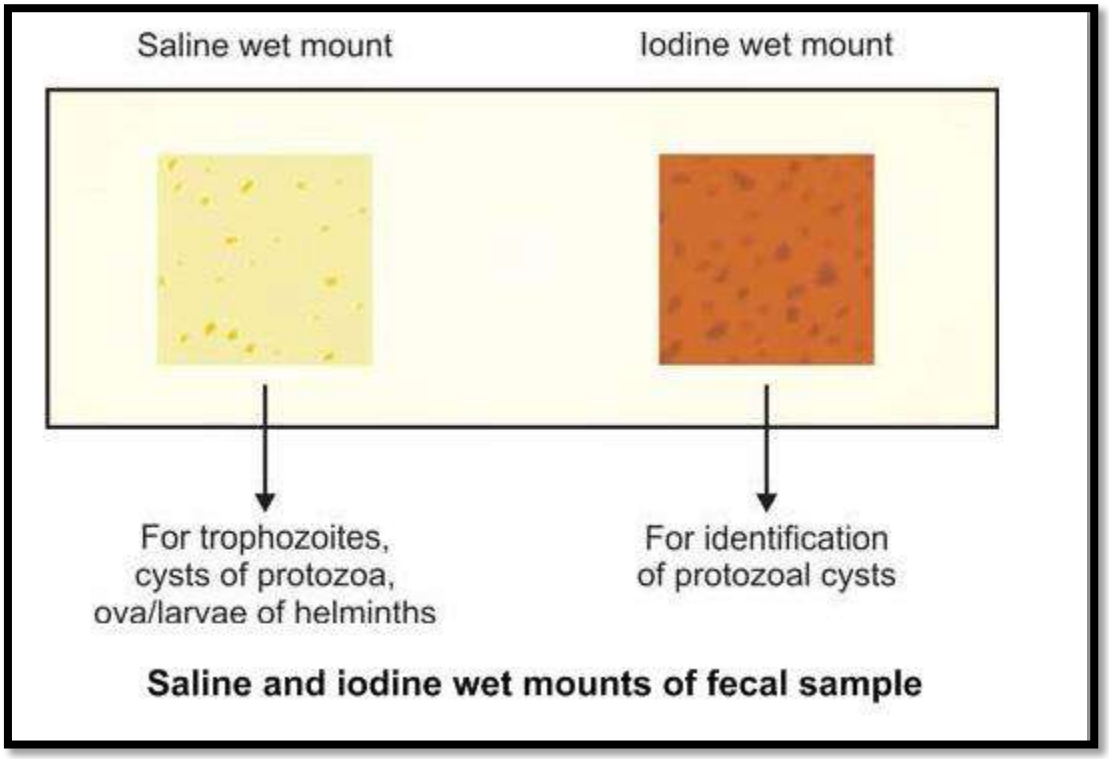
7- Parasite: Stools may contain adult helminthes. Nematodes like Ascaris are easily visible as their size is large. Hook worms and Proglottids of cetodes may also present. These may be visible to the naked eye.

b. MICROSCOPIC EXAMINATION OF STOOL:

The laboratory diagnosis of most parasitic infections is by the demonstration of ova of the parasite in the stools of the infected person. The stool is collected in a clean container. The stool can be examined by the following techniques: wet mounts examination and iodine examination.

Saline wet mount examination: the stool is emulsified in normal saline and a large drop is placed on a glass slide and is then covered with a cover slide. Then examined under a light microscope, it is important to examine specimen under 10X objective lens at first to observe large molecules, cells, ova and helminthes, then to the 40X objective to complete the test. It is preferable to keep the condenser down and the intensity of the light low for proper visualization of the ova and cysts. The thickness of the film should be such that one is able to see the printed letters of the newspaper through it.

Iodine examination: Iodine preparation leads to better visualization of morphological details of ova and cysts as it stains the glycogen in them. However it has the disadvantage that the live trophozoites of *Entamoeba histolytica* and other live parasites cannot be seen as the iodine kills them. The examination instructions in normal saline must be followed the same in iodine test.



Saline and iodine wet mounts of fecal sample

Microscopic examination include the following:

(1) Pus cells: Observed in stool the same procedure as in urine.

(2) RBCs: Observed in stool the same procedure as in urine.

(3) Monilia: Observed in stool the same procedure as in urine.

(4) Protozoa: (a) *Entamoeba histolytica*: to investigate the vegetative phase (trophozoite) and cyst, causing amoebic dysentery disease. **(b)** *Entamoeba coli*: trophozoite + cyst *Note:* - most of children diarrhea less than 2 years cause by *Entamoeba coli*. **(c)** *Giardia lamblia*, trophozoite + cyst, Cause watery diarrhea disease in children, especially. **(d)** *Balantidium coli*, trophozoite + cyst, causing Balantidiasis in colon.

(5) Worms : (a) *Enterobius vermicularis* (pinworm): investigating the eggs that are of convex and flat surface and a pointed end. **(b)** *Ascaris lumbricoides:* investigating for eggs which characterized by the content of granular yellow to Brown irregular albumin membrane. **(c)** Hookworm (*Ancylostoma duodenale)*: investigating the eggs where the egg yolk is divided and surrounded by a thin membrane. **(d)** Tapeworms, (Taenia *solium)*: investigating the worm pieces called (gravid segments or Proglottids) that comes out with the feces. **(e)** *Schistosoma mansoni*: Investigating the eggs distinct by lateral spin.

c. CHEMICAL EXAMINATION OF STOOL:

1- pH: the pH of stools is acidic in amoebic dysentery and is alkaline in bacillarydysentery.

2- Occult blood: presence of blood in feces which is not apparent on gross inspection and which can be detected only by chemical tests is called as occult blood. Causes of occult blood present in a number of diseases including malignancy of the gastrointestinal tract.

The reagents used are:

1- Benzidine reagent: development of blue colour is indicative of presence of occult blood in the stool specimen.

2- Orthotolidine: development of green colour Benzidine test is also highly sensitive and false-positive reactions are common. Since bleeding from the lesion may be intermittent, repeated testing may be required.

Causes of False-positive Tests:

1. Ingestion of peroxidase-containing foods like red meat, fish, poultry, turnips, horseradish, cauliflower, spinach, or cucumber. Diet should be free from peroxidase-containing foods for at least 3 days prior to testing.

2. Drugs like aspirin and other anti-inflammatory drugs, which increase blood loss from gastrointestinal tract in normal persons.

Causes of False-negative Tests:

1. Foods containing large amounts of vitamin C.

2. Conversion of all hemoglobin to acid hematin (which has no peroxidase-like activity) during passage through the gastrointestinal tract.

d. STOOL CULTURE:

How to take stool samples:

1. Specimen of stool (at least 4 ml or 4 cm^3) is collected in a clean, dry, container with a tightly fitting lid.

2. Do not let sample stand for a long period of time (so as not to die eccentric parasitic and inspection preferably within an hour of taking the sample).

3. Early morning is the best sample because the stool here all night and the chances of the emergence of complex parasites and eggs are the largest.

4. For children prefer to urinate first before taking a stool sample so as not to mix urine with stool sample.

5. Stool should not be contaminated with urine, water, soil, or menstrual blood. Urine and water destroy trophozoites; soil will introduce extraneous organisms and also hinder proper examination.

6. Patient's name, date, time and number must be labeled on the sample container.

7. Stools should be examined as early as possible after receipt in the laboratory (preferably within 1 hour of collection). If delay in examination is anticipated, sample may be refrigerated because Parasites are best detected in warm, freshly passed stools.

8. Patient must not use laxatives prior collecting stool sample.

9. Patient must refrain from taking certain medicines before the test such as: pH medications, diarrhea medications, anti - parasite drugs, antibiotics.

10. The patient must wear gloves before handling a stool sample in order to avoid transmission or use the tool to move the sample in the container and don't take a stool sample base of the bathroom (toilet) floor.

11. The patient must wash his hands thoroughly after taking the sample and the sample water or soap does not mix.

Procedure:

Stool is cultured by taking a sample by loop and cultured on different types of culture media according to the type of bacteria or diagnosis of case investigated as follows:

1- It is cultured on thiosulfate citrate bile salts sucrose agar media if the patient is suspected of cholera infection.

2- It is cultured on Lowenstein-Jensen medium for *Mycobacterium tuberculosis* if the person suspected of gastrointestinal tuberculosis.

3- It is cultured on blood agar if suspected of infection with *Staphylococcus aureus* which it is blood hemolytic.

4- It is cultured on MacConkey agar medium to detect lactose fermentation bacteria in pink color colonies include (*E .coli*, *Klebsiella* and *Enterobacter*), but if it's not lactose fermenter, it is either *Proteus* which is identified by (diffusion phenomenon), or *pseudomonas* which is identified by (pyocynin test) and to distinguish between *Shigella* and *Salmonella*, by using serological and biochemical test.

الأسبوع الثامن

الهدف التعليمي: التعرف على السائل المنوي وطرق فحصه.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Seminal Fluid**

Semen (or seminal fluid) is a fluid that is emitted from the male genital tract and contains sperms, sugar and protein substances that are capable of fertilizing female ova. Semen analysis, include two major tests:

a- Macroscopic or Physical Examination: that includes; volume, color, viscosity (time to liquefaction), and pH.

b- Microscopic Examination: that includes; sperm count, motility and vitality, morphology, and proportion of white cells.

a- Macroscopic or Physical examination:

Physical Examination is carried out after liquefaction of semen that occurs usually within 20-30 minutes of ejaculation.

1) Volume:

Volume of ejaculated semen sample should normally be more than 2 ml. It is measured after the sample has liquefied. Volume less than 2.0 ml is abnormal, and is associated with low sperm count. Excess Volume more than 5 ml. could also mean diluted amount of sperm present in ejaculum.

2) Color:

Normal semen is viscous and opaque-white (milky) or opaque gray-white in color. After prolonged abstinence , it appears slightly yellow. But In case of an inflammatory purulent appears yellow. Sometimes appear brown in color in cases of bleeding from a blood capillaries.

3) Viscosity:

Normal semen is thick and viscous immediately following ejaculation, it becomes liquefied within (10 to 30) minutes at (37 °C) by the action of proteolytic enzymes secreted by prostate

and turned into a watery consistency, helps sperm to move. It is considered abnormal if liquefaction does not occur within 60 minutes.

The viscosity of the sample is assessed by filling a pipette with semen and allowing it to flow back into the container. Normal semen will fall drop by drop. If droplets form 'threads' more than 2 cm long, then viscosity is increased.

Increased semen viscosity affects sperm motility and leads to poor invasion of cervical mucus; (it results from infection of seminal vesicles or prostate).

Report is written as follows:

Liquefaction within 30 min. at 37 °C

or in case of delayed liquefaction is written:

Viscous after 1 hr. incubation at 37 °C

4) pH:

A drop of liquefied semen is spread on pH paper (of pH range 6.4-8.0) and pH is recorded after 30 seconds. Normal pH is (7.2 to 8.0) after 1 hour of ejaculation.

The portion of semen contributed by seminal vesicles is alkaline, while portion from prostate is acidic.

1- Low pH (< 7.0) with absence of sperms (Azoospermia) suggests obstruction of ejaculatory ducts or absence of vas deferens.

2- High pH is usually associated with low semen volume [as most of the volume is supplied by seminal vesicles (no prostatic fluid)].

b- Microscopic Examination:

The most important test in semen analysis for infertility is microscopic examination of the semen, which include:

1) Sperm Count:

The sperm count is done after liquefaction and the total number of spermatozoa is reported in millions/ml or (106/ml), and there are two methods for sperm count**:**

- Direct method:

This method is done by taking a drop of semen on clean slid and covered with a cover slide and immediately examined under 40× objective lens and each five sperm per microscopic field represents one million sperm per ml or cm^3.

- Haemocytometer:

In this method sperm count is done after liquefaction in a counting chamber **(Haemocytometer)** following dilution with diluting fluid and the total number of spermatozoa is reported in millions/ml or (106/ml).

Note: Semen specimen is incubated at 37°C after collection to insure liquefaction.

Diluting fluid:

Sodium bicarbonate formalin (1 ml formalin + 5 gm. sodium bicarbonate)

Procedure:

1. Semen is diluted 1:20 with diluting fluid, (take 1 ml of liquefied semen in a graduated tube and fill with diluting fluid to 20 ml mark. Mix well), or by using WBC diluting pipette just the same way in WBC total count, and a coverslip is placed over the counting chamber.
2. Counting chamber is filled with the well-mixed diluted semen sample using a Pasteur pipette. The chamber is then sit for 10-15 minutes for spermatozoa to settle.
3. The chamber is placed on the microscope stage. Using the 40× objective and iris diaphragm lowered sufficiently to give sufficient contrast, number of spermatozoa is counted in 4 large corner squares. Spermatozoa whose heads are touching left and upper lines of the square should be counted considered as 'belonging' to that square.
4. Sperm count per ml is calculated as follows: *Sperm count = Sperms counted × correction factor × 1000 ÷ Number of squares counted × Volume of 1 square*
5. Normal sperm count is ≥ 20 million/ml (i.e. ≥ 20 × 106/ml).
6. Sperm count < 20 million/ml may be associated with infertility in males.

Few millions less than 10 called Oligospermia. Zero sperm count (absence of any sperm in the semen) called Azoospermia.

Count / ml = N/4 *10 *1000* 20

N :

The total count in 4 squares

10 :

Volume factor

1000 :

Generally the sperm count by ml or cm^3 so multiply by 1000 to convert mm3 to ml or cm^3

20 :

dilute factor

Count /ml = N * 50,000

Some common abnormal results in semen count:

1. **Azoospermia**: complete absence of sperm from the ejaculate, present in about 1% of all men and 10%-15% of infertile men.

2. **Aspermia**: complete absence of seminal fluid emission upon ejaculation.

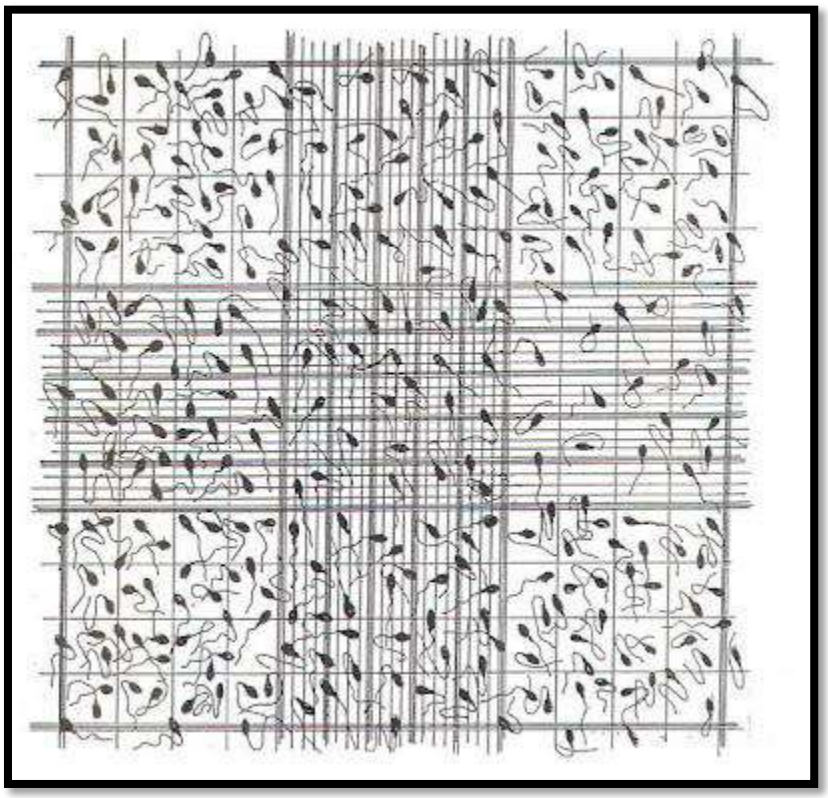
3. **Oligospermia**: low sperm count, defined by the World Health Organization (WHO) as concentrations less than 15 million sperm/ml.

Oligospermia is further classified as:

(a) Mild: concentrations 10-15 million sperm/ml.

(b) Moderate: concentrations of 5-10 million sperm/ml.

(c) Severe: less than 5 million sperm/ml.



Sperm count in haemocytometer

2) Motility or Movement:

The first laboratory assessment of sperm function in a wet preparation is sperm motility (ability of the sperms to move). Sperm motility is essential for penetration of cervical mucus, traveling through the fallopian tube, and penetrating the ovum. Only those sperms having rapidly progressive motility are capable of penetrating ovum and fertilizing it.

For a normal result, more than 50 percent of sperm must move normally an hour after ejaculation, the sperm motility divided into:

a- Active; rapidly progressive spermatozoa (moving fast forward in a straight line),

b- Sluggish; Slowly progressive spermatozoa (slow linear or non-linear, i.e. crooked or curved movement),

c- Non-progressive spermatozoa (movement of tails, but with no forward progress).

d- Immotile spermatozoa (no movement at all) (WHO criteria).

Sperms of grades (c) and (d) are considered to be poorly motile (Asthenospermia).

Procedure:

A drop of semen is placed on a glass slide, covered with a coverslip that is then ringed with petroleum jelly to prevent dehydration, and examined under 40× objective. At least 200 spermatozoa are counted in several different microscopic fields. Result is expressed as a percentage (%).

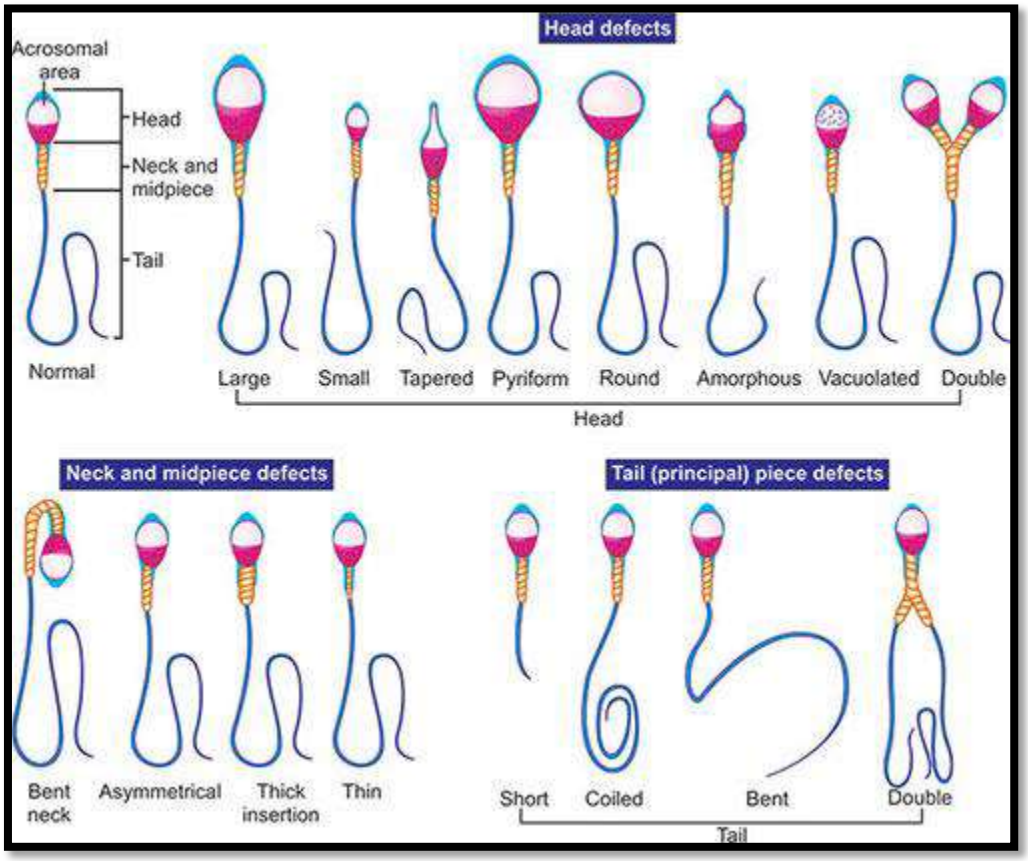
Normal percentage is ≥ 50% of sperms show rapid progressive and slow progressive motility.

3) Sperm Morphology:

A spermatozoa consists of three main components: head, neck, and tail. Tail is further subdivided into midpiece (principle) piece, and end piece. The defects in morphology that are associated with infertility in males include:

- Defective mid-piece (causes reduced motility).
- Incomplete or absent acrosome (causes inability to penetrate the ovum).
- Giant head (defective DNA condensation).

Normal results; more than 50% of spermatozoa should be normal morphology (WHO, 1999).



Procedure:

A smear is prepared by spreading a drop of seminal fluid on a glass slide, stained, and percentages of normal and abnormal forms of spermatozoa are counted. The staining techniques used are hematoxylin-eosin, and rose bengal-toluidine blue stain. At least 200 spermatozoa should be counted under oil immersion. Percentages of normal and abnormal spermatozoa should be recorded.

Teratozoospermia, also known as Teratospermia, is a semen alteration in which there is a large number of spermatozoa with abnormal morphology or (it is a condition characterized by the presence of sperm with abnormal morphology that affects fertility in males).

4) Proportion of White Cells:

In microscopic examination also investigating the presence of white blood cells (Pus cells) in cases of; Gonorrheal infection, inflammation of the prostate, and sometimes a parasite like *Trichomonas vaginals* also found which is transmitted through sexual intercourse (S.T.D; Sexually transmitted disease).

Round cells on microscopic examination may be white blood cells or immature sperm cells.

It is very important to differentiate between them by using a special stain (peroxidase or papanicolaou) is required to differentiate between them.

- Presence of large number of immature sperm cells indicates spermatogenesis dysfunction at the testicular level.
- Presence of White blood cells more than (1 million/ml) indicate presence of infection, and semen culture and sensitivity is required.

Culture of Seminal Fluid:

Like any other body secretions semen specimens are cultured on the following media:

1- Blood Agar.

2- Chocolate agar.

3- MacConkey agar.

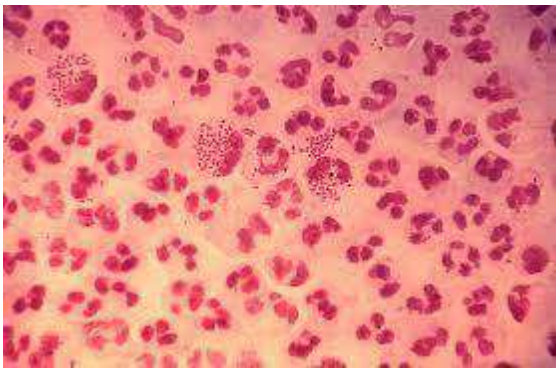
Culture is done at first and other tests handled after to avoid contamination by streaking a loop on plates.

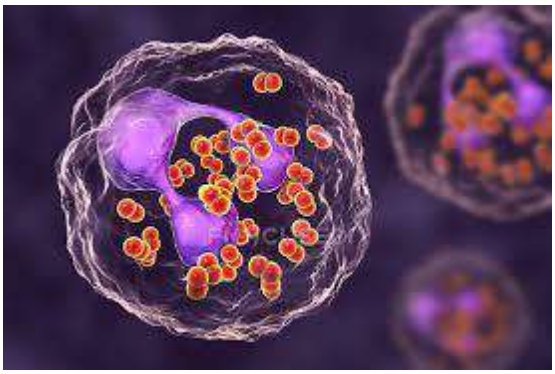
Blood Agar and Chocolate agar are incubated anaerobically in candle jar provided with CO_2 ratio of (5-10 %) for anaerobic bacteria, and MacConkey agar is incubated aerobically for aerobic bacteria.

The most important types of bacteria that are found in seminal fluid are:

- *Neisseria gonorrhoeae* (intracellular Gram neg. diplococci; shown in figure below).
- *Staphylococcus aureus.*
- *Mycoplasma.*
- Coliforms.

Antibiotic sensitivity test is performed after culture on identified bacterial growth to determine sensitive and resistant antimicrobial drugs.





الأسبوع التاسع

الهدف التعليمي: التعرف على تقنية التلازن.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Agglutination technique**

Agglutination technique detect antibody or antigen and involve agglutination of bacteria, red cells, or antigen- or antibody coated latex particles. Such tests rely on the bivalent nature of antibodies, which can cross-link particulate antigens.

Steps of Agglutination:

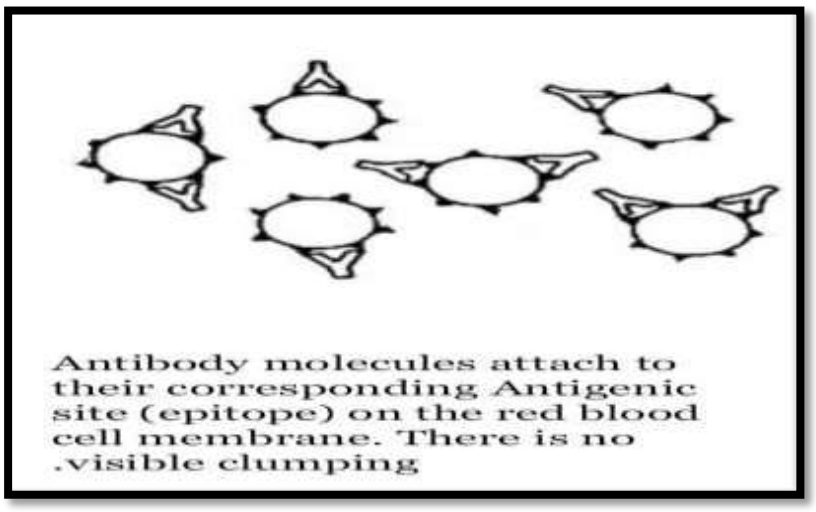
-Primary phenomenon (sensitization).

-Lattice formation (aggregation stage).

-Tertiary phenomenon.

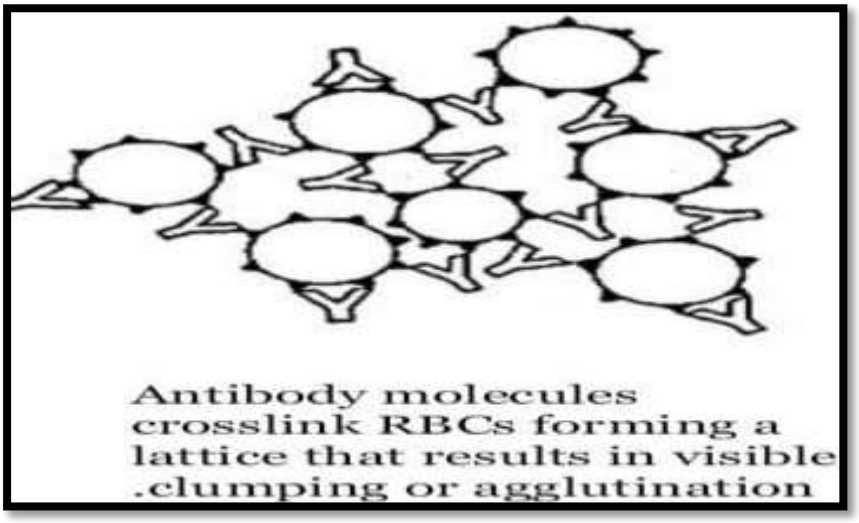
1- Primary phenomenon (sensitization):

Involve antigen-antibody combination through single antigenic determination on the particle.



2- Lattice formation (aggregation stage):

Represent the sum of interaction between antibody and multiple antigenic determinants on a particle dependent on environmental conditions as well as the concentration of antigen and antibody.



3- Tertiary phenomenon:

Reaction not visible, detected by effect of reaction on tissues or cells.

Uses of Agglutination:

1- Aid in the identification, by means of known antisera(serum containing antibodies specific for a given antigen),microorganisms cultured from clinical specimens.

2- Help estimate the titer of antibacterial agglutination in the serum of patients with unknown disease.

Types of Agglutination:

a- Direct agglutination (active).

b- Indirect agglutination (passive).

c- Reverse agglutination.

d- Hemagglutination.

e- Hemagglutination inhibition.

f- Coagglutination.

Qualitative Agglutination:

Agglutination test can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen.

Quantitative Agglutination:

Agglutination test can also be used to quantitate the level of antibodies to particulate antigens. In this test one makes serial dilutions of a sample to be tested for antibody and then adds a fixed number of red blood cells or bacteria or other such particulate antigen and determines the maximum dilution, which gives agglutination. The maximum dilution that gives visible agglutination is called the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination. This can be done using a micro titer plate.

Methods of Agglutination:

Slide agglutination (rapid): add a drop of antiserum , mix with antigen and rock slide for approx. 1minute.

Tube agglutination (slow): standard quantitive method for determination of antibodies . Routinely employed in diagnosis of different types of viruses and bacteria.

Advantages of Agglutination:

1- Low individual test cost.

2- Ability to obtain semi quantitative results.

3- Short time to obtain result.

4- Don’t need expensive instrument.

5- Agglutination of insoluble native antigens or antigen-coated particles simple to read with or without the aid of a microscope.

6- Increased degree of sensitivity.

7- Great variety of detectable substances.

8- If the sample contain micro-organisms, it does not need to be viable.

الأسبوع العاشر

الهدف التعليمي: التعرف على اختبار الاليزا.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Enzyme-linked immunosorbent assay (ELISA)**

Enzyme-linked immunosorbent assay (ELISA) test is the most widely used type of immunoassay. ELISA is a rapid test used for detecting or quantifying antibody (Ab) against viruses, bacteria and other materials or antigen (Ag). ELISA is so named because the test technique involves the use of an enzyme system and immunosorbent.

ELISA test is being increasingly used in the detection of antigen (infectious agent) or antibody due to its simplicity and sensitivity. It is as sensitive as radioimmunoassay (RIA) and requires only microlitre quantities of test reagents. It has now been widely applied in detection of a variety of antibody and antigens such as hormones, toxins, and viruses.

Salient Features of ELISA Test:

1. ELISA test has high sensitivity and specificity.

2. The result of quantitative ELISA tests can be read visually

3. A large number of tests can be done at one time . ELISAs are designed specifically for screening large numbers of specimens at a time, making them suitable for use in surveillance and centralized blood transfusion services

4. Reagents used for ELISA are stable and can be distributed in district and rural laboratories but as ELISAs require sophisticated equipment and skilled technicians to perform the tests, their use is limited to certain circumstances.

Materials needed in ELISA Test:

1. ELISA Readers: need to have appropriate filter (650 nm and 450 nm).

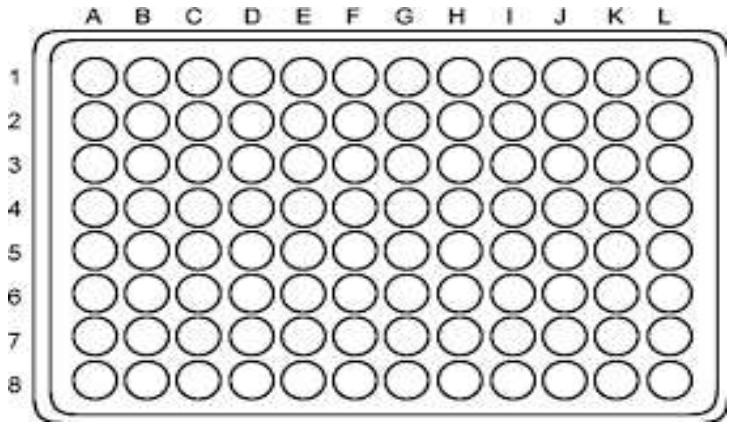
2. Pipette: are available as fixed as well as adjustable volume as well as single channel and multi-channel.

3. Washing system: it can be manual system that washes one row or column at a time or semi-automated systems that wash one strip or plate at a time or fully automated systems that can process multiple plates

4. Reagents: needed for the testing concluded in the kit (coated plates , sample diluents , controls, wash concentrate, conjugate, substrate, stop solution).

Principle:

ELISAs are typically performed in 96-well polystyrene plates. The serum is incubated in a well, and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface. After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer. To detect the bound antibodies or antigens, a secondary antibodies that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well. After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a color. This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of color/ optical density is measured at 450 nm. The intensity of the color gives an indication of the amount of antigen or antibody.



Types of ELISA:

Frequently there are four types of ELISA on the basis of binding structure between the Antibody and Antigen.

1. Direct ELISA.

2. Indirect ELISA.

3. Sandwich ELISA.

4. Competitive ELISA.

1. Direct ELISA:

For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme. This detection method is a good option if there is no commercially available ELISA kits for your target protein.

2. Indirect ELISA:

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form colored products. The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.

3. Sandwich ELISA:

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.

4. Competitive ELISA:

This test is used to measure the concentration of an antigen in a sample. In this test, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to the microtitre well which is coated with antigen. The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well. The higher the concentration of antigen in the sample, the lower the absorbance.

Application of ELISA:

1. Presence of antigen or the presence of antibody in a sample can be evaluated.

2. Determination of serum antibody concentrations in a virus test.

3. Used in food industry when detecting potential food allergens.

4. Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common,

colds, cholera, STD etc.

الأسبوع الحادي عشر

الهدف التعليمي: التعرف على اختبار المناعة الاشعاعية.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Radioimmunoassay (RIA)**

Radioimmunoassay (RIA), a highly sensitive laboratory technique used to measure minute amounts of substances including antigens, hormones, and drugs present in the body. The substance or antigen (a foreign substance or pathogen in the body that causes antibody production by the B lymphocytes of the body) to be measured is injected into an animal, causing it to produce antibodies. Serum containing the antibodies is withdrawn and treated with a radioactive antigen and later with a nonradioactive antigen. Measurements of the amount of radioactivity are then used to determine the amount of antigen present.

Principle and Procedure of RIA:

Radioimmunoassay combines the principles of radioactivity of isotopes and immunological reactions of antigen and antibody, hence the name. The principle of RIA is primarily based on the competition between the labelled and unlabeled antigens to bind with antibody to form antigen-antibody complexes (either labeled or unlabeled).

The unlabeled antigen is the substance (say some hormone or virus) to be determined. The antibody to it is produced by injecting the antigen to a goat or a rabbit. The specific antibody (Ab) is then subjected to react with unlabeled antigen in the presence of excess amounts of isotopically labelled (125I) antigen (Ag+) with known radioactivity. There occurs a competition between the antigens (Ag+ and Ag) to bind the antibody. Certainly, the labeled Ag+ will have an upper hand due to its excess presence.

As the concentration of unlabeled antigen (Ag) increases the amount of labelled antigen-antibody complex (Ag+-Ab) decreases. Thus, the concentration of Ag+- Ab is inversely related to the concentration of unlabeled Ag i.e., the substance to be determined.

This relation is almost linear. The labeled antigen-antibody (Ag+-Ab) complex is separated by precipitation. The radioactivity of 125I present in Ag+-Ab is determined. To determine the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen (antigen not bound to Ab), and the radioactivity in the precipitate is measured .

Advantages of RIA:

1- Radioimmunoassay is very sensitive technique used to measure concentrations of antigens without the need to use a bioassay.

2- It can measure one trillionth (1012) of a gram of material per milliliter of blood.

3- It is structurally specific as antigen antibody reaction are highly specific.

4- It is indirect method of analysis.

Uses of RIA:

1- Narcotics (drugs) detection.

2- Blood bank screening for the hepatitis (a highly contagious condition) virus.

3- Early cancer detection.

4- Measurement of growth hormone levels.

5- Tracking of the leukemia virus.

6- Diagnosis and treatment of peptic ulcers.

7- Research with brain chemicals called neurotransmitters.

Applications:

1- RIA is used in the estimation of substances that exhibit antigenic property either as such or by chemical modification.

2- Substances that are estimated by RIA are hormones , tumor markers (peptides , proteins , steroids, etc.), vitamins, drugs, etc.

3- Radioimmunoassay has applications in the diagnosis of hormonal disorders, cancers and therapeutic monitoring of drugs.

4- It is also useful in biomedical research .

الأسبوع الثاني عشر

الهدف التعليمي: التعرف على تقنية التألق المناعي.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Immunofluorescence Technique**

Introduction:

Immunofluorescence is histochemical laboratory staining technique used for demonstrating the presence of Antibodies bound to Antigen in tissues or circulatory body fluids. Immunofluorescence is the labeling of antibodies or antigens with fluorescent dyes.

They are permit early diagnosis, treatment ,and subsequent monitoring of disease activity in patients . Fluorochromes are dyes that absorb ultra-violet rays and emit visible light. This process is called fluorescence. The fluorochromes commonly used in immunofluorescence are fluorescein isothiocyanate (FITC) (green) and tetra methyl rhodamine isothiocyanate (TRITC) (red). Fluorescent techniques involve the emission of light of one color/wavelength and a low energy level from a substance being irradiated with light of a different wavelength

The antibody is linked with fluorescein isothiocyanate (FITC) via a thiocarbamide linkage without destroying its capacity to react with the corresponding antigen.

There are three basic types of immunofluorescence techniques:

1. Direct immunofluorescence **(**DIF).
2. Indirect immunofluorescence (IIF).
3. Microimmunofluorescence (MIF).

Direct immunofluorescence:

This is a one-step histological staining procedure for identifying *in vivo* antibodies that are bound to tissue antigens. This technique is used to detect antigen in clinical specimens using specific fluorochrome labeled antibody.

The steps involved are:

1- Fixation of smear on the slide.

2- Treating with labeled antibody.

3- Incubation, washing to remove unbound excess labeled antibody visualization under fluorescent microscope. When viewed under fluorescent microscope, the field is dark and areas with bound antibody fluoresce green.

4- Ag is fixed on the slide.

5- Fluorescein labeled Ab's are layered over it.

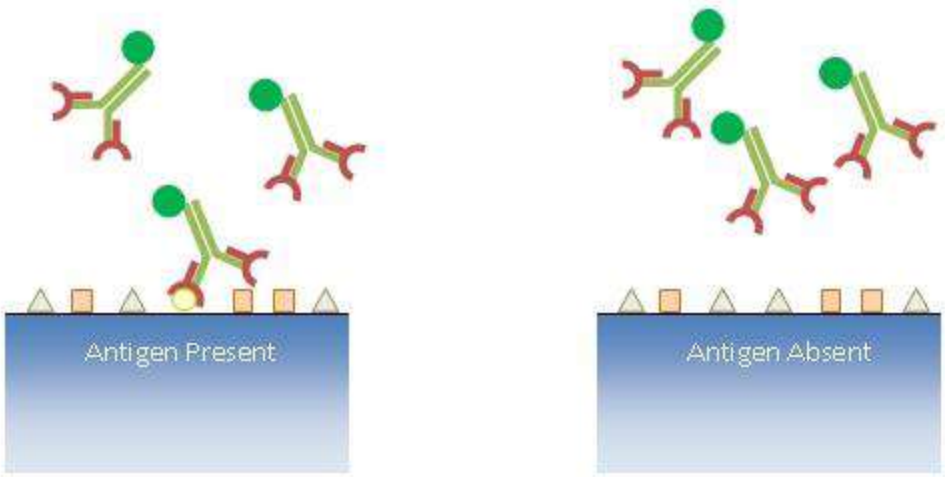
6- Slide is washed to remove unattached Ab's.

7- Examined under UV light in an fluorescent microscope.

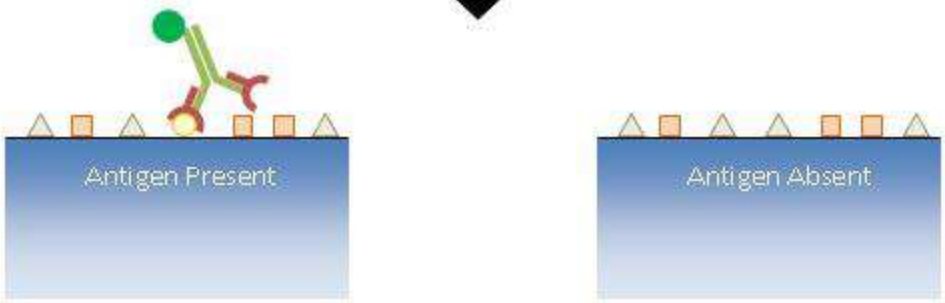
8- The site where the Ab attaches to its specific Ag will show apple green fluorescence.

9- This technique can be used to detect pathogens or their Ag's in tissues or in pathological samples. viral, parasitic, tumor antigens from patient specimens or monolayer of cells.

10- Another application is identification of anatomic distribution of an antigen within a tissue or within compartments.



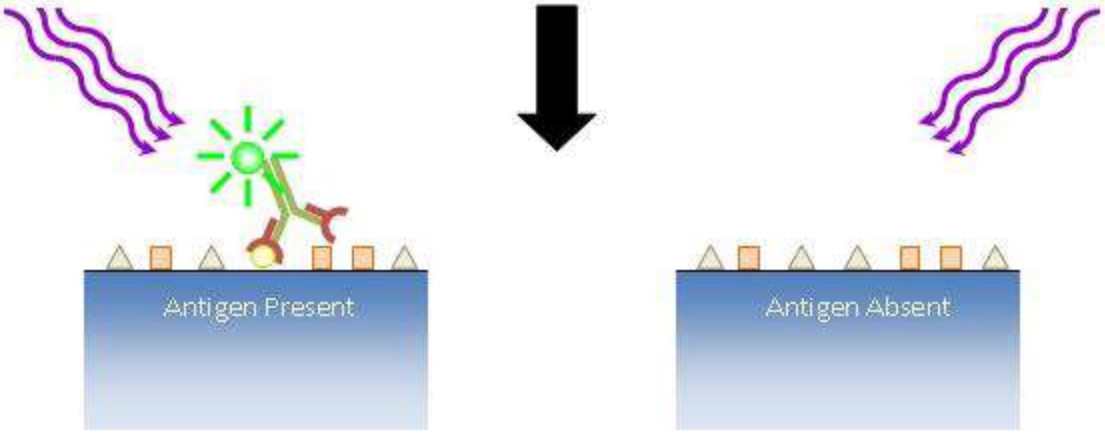
Treatment with labeled antibody



Unbound antibody washed away

UV Light

UV Light

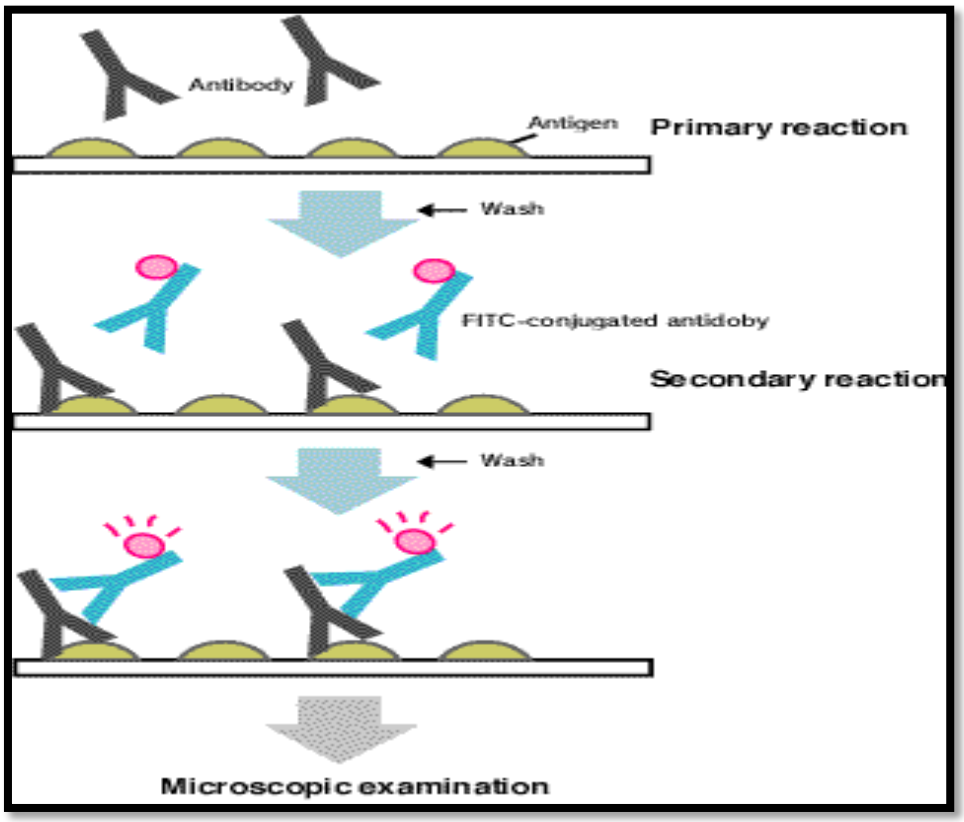


Fluorescence observed where antigen is located

Indirect immunofluorescence:

Is employed to detect antibodies in patient serum. The antigen on smear are made to react with specific unlabeled antibody and washed. The unbound antibody gets washed off adding another antibody. The second antibody is labeled anti-gamma globulin antibodies. This antibody binds to Fc portion of first antibody and persists despite washing. The presence of the second antibody is detecting by observing under fluorescent microscope.

It is often used to detect auto antibodies .Commonly used in the detection of anti-nuclear antibodies (ANA) found in the serum of patients with SLE. irIndect test is a double-layer technique. The unlabeled antibody is applied directly to the tissue substrate Treated with a fluorochrome-conjugated anti-immunoglobulin serum.



Microimmunofluorescence:

This is a serological technique employed to detect antibodies in patient serum. It works on the same principle as that of indirect immunofluorescence but is performed on Teflon slides with many wells dotted with antigens. This technique is used in the serodiagnosis of Q fever, Mediterranean spotted fever, Detection of IgG, IgA and IgM Antibodies to Chlamydia, toxoplasmosis, epidemic typhus etc.

الأسبوع الثالث عشر

الهدف التعليمي: التعرف على تفاعل سلسلة البلمرة.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Polymerase chain reaction (PCR)**

Polymerase chain reaction (PCR) is a common laboratory technique used to amplify or make millions of copies of a particular region of DNA. This DNA region can be anything the experimenter is interested in. For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects. It was originally invented by Kary Mullis in 1985 and got the Nobel Prize in 1993.

Principle:

The basic principle of PCR is that the double stranded DNA molecule, when heated to a high temperature, separate yielding two single-stranded DNA molecules. The single stranded DNA molecules can easily be copied with the help of a DNA polymerase and nucleosides resulting in the duplication of original DNA molecules. By repeating these events, multiple copies of the original DNA molecule can be generated.

Requirements:

i) A thermal cycler (an instrument having a microprocessor-controlled temperature cycling).

ii) DNA segment to be amplified.

iii) Two primers, which are oligonucleotides (about 10-18 nucleotides long), oriented with their ends facing each other so that DNA synthesis can occur between them.

iv) The enzyme Taq polymerase (a DNA polymerase) which is stable at high temperature.

v) MgCl2.

vi) dNTPs (deoxy nucleoside triphosphate: dATPs, dTTPs, dGTPs, dCTPs).

Procedure:

The DNA, from which a segment is to be amplified, is mixed with an excess of the two primer molecules, all the four kinds of dNTPs, MgCl2 and Taq polymerase in a reaction mixture. The DNA segment is amplified involving the following 3 steps:

i) Denaturation: The reaction mixture is heated to a high temperature (94-96ºC) so that the DNA molecule is denatured i.e. the two strands of DNA duplex get separated. Each strand of the target DNA then acts as a template for DNA synthesis.

ii) Annealing: The mixture is then cooled by lowering the temperature upto 55-65ºC. At this temperature, the two primers anneal to each of the single-stranded template DNA. Annealing occurs due to presence of complementary sequences located at the 3' ends of the template DNA.

iii) Extension: In this step, the temperature is so adjusted that the Taq polymerase becomes active. Synthesis of new DNA strand begins in between the primers, dNTPs and Mg2+. The optimum temperature for this polymerization is kept at 72ºC.

The next PCR amplification cycle begins as soon as all the stages of previous cycle end. During PCR operation, the extension product of one cycle serve as a template for subsequent cycles and each time the amount of DNA doubles. Thus, a single template molecule of DNA generates 2n molecules at the end of n cycles.

Applications:

PCR is useful in every aspect of modern biology including-molecular biology, genetic engineering, infectious and parasitic disease diagnosis, human genetic disease diagnosis, forensic validation, DNA fingerprinting, plant and animal breeding and environmental monitoring.

الأسبوع الرابع عشر

الهدف التعليمي: التعرف على تفاعل سلسلة البلمرة في الوقت الحقيقي.

مدة المحاضرة: ساعات (ساعتان نظري+ 4 ساعات عملي).

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Real-Time (PCR)**

Real-Time PCR is a technique used to monitor the progress of a PCR reaction in real-time.

At the same time, a relatively small amount of PCR product (DNA, cDNA or RNA) can be quantified. Real-Time PCR is based on the detection of the fluorescence produced by a reporter molecule which increases, as the reaction proceeds. Real-Time PCR is also known as a quantitative polymerase chain reaction (qPCR), which is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR).

qPCR is a powerful technique that allows exponential amplification of DNA sequences.

A PCR reaction needs a pair of primers that are complementary to the sequence of interest. Primers are extended by the DNA polymerase. The copies produced after the extension, so-called amplicons, are re-amplified with the same primers leading thus to exponential amplification of the DNA molecules. After amplification, however, gel electrophoresis is used to analyze the amplified PCR products and this makes conventional PCR time consuming; since the reaction must finish before proceeding with the post-PCR analysis. Real-Time PCR overcomes this problem.

The term “real-time” denotes that it can monitor the progress of the amplification when the process is going on in contrast to the conventional PCR method where analysis is possible only after the process is completed.

Principle of Real-Time PCR:

This same principle of amplification of PCR is employed in real-time PCR. But instead of looking at bands on a gel at the end of the reaction, the process is monitored in “real-time”. The reaction is placed into a real-time PCR machine that watches the reaction occur with a camera or detector.

Although many different techniques are used to monitor the progress of a PCR reaction, all have one thing in common. They all link the amplification of DNA to the generation of fluorescence which can simply be detected with a camera during each PCR cycle. Hence, as the number of gene copies increases during the reaction, so does the fluorescence, indicating the progress of the reaction.

Steps of Real-Time PCR (Protocol):

The working procedure can be divided into two steps:

a. Amplification:

1- Denaturation:

High temperature incubation is used to "melt" double- stranded DNA into single strands and loosen secondary structure in single-stranded DNA. The highest temperature that the DNA polymerase can withstand is typically used (usually 95°C). The denaturation time can be increased if template GC content is high.

2- Annealing:

During annealing, complementary sequences have an opportunity to hybridize, so an appropriate temperature is used that is based on the calculated melting temperature (Tm) of the primers(5°C below the Tm of the primer).

3- Extension:

At 70-72°C, the activity of the DNA polymerase is optimal, and primer extension occurs at rates of up to 100 bases per second. When an amplicon in real-time PCR is small, this step is often combined with the annealing step using 60°C as the temperature.

b. Detection:

The detection is based on fluorescence technology. The specimen is first kept in proper well and subjected to thermal cycle like in the normal PCR. The machine, however, in the Real Time PCR is subjected to tungsten or halogen source that lead to fluoresce the marker added to the sample and the signal is amplified with the amplification of copy number of sample DNA. The emitted signal is detected by an detector and sent to computer after conversion into digital signal that is displayed on screen. The signal can be detected when it comes up the threshold level (lowest detection level of the detector).

Advantages of Real-Time PCR:

1- It gives a look in to the reaction that is help to decide which reactions have worked well and which have failed.

2- The efficiency of the reaction can be precisely calculated.

3- There is no need to run the PCR product out on a gel after the reaction as the melt curve analysis serve the purpose.

4- The real-time PCR data can be used to perform truly quantitative analysis of gene expression. In comparison, old fashioned PCR was only ever semi-quantitative at best.

5- Faster than normal PCR.

6- Less complexity at the quantification of sample etc.

Applications of Real-Time PCR:

- Gene expression analysis.
- Cancer research.
- Drug research.
- Disease diagnosis and management.
- Viral quantification.
- Food testing.
- GMO food.
- Animal and plant breeding.
- Gene copy number.

الأسبوع الخامس عشر: مراجعة