المحاضرة الاولى

Laboratory instruments

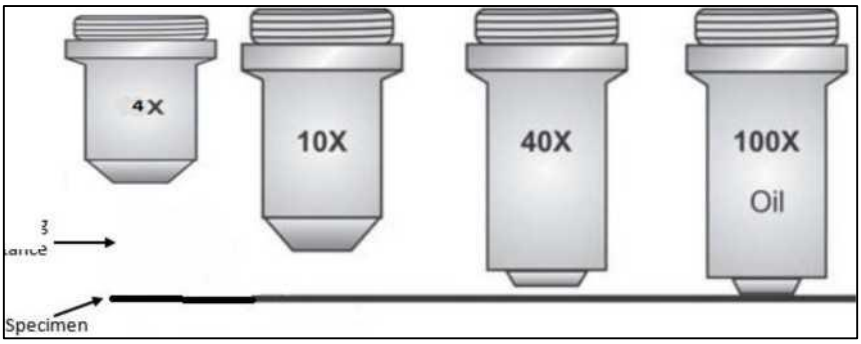
" Laboratory instrument" is a general term for all types of instruments, vessels, and other tools needed for operations in laboratories. Analytical lab instruments included a wide range of instrumentation whose principle purpose is to qualitatively and quantitatively analyze samples. Most devices are expensive and sensitive and are affected by the environment such as heat and humidity, so it must be maintained, Periodically clean and maintain them also calibration for each device to ensure the sensitivity of the device to obtain accurate results. The instrument must have a long life and provide safety for the user. Laboratory a room or building are equipped with instruments ,tools, material &other items using for tests , experiments ,analysis & for teaching, or a place where chemicals or medicines are produced. The laboratory contains Bench and Locker for storing materials also the tools and instruments in the laboratory vary according to the purpose of the laboratory such as microbiology lab, physiology lab, immunology lab, human biology lab, histology, anatomy lab, chemistrylab and genetics laboratory ,each laboratory contains special instruments that we study in detail.

The Microscope

Microscopes is an instrument used to see objects that are too small to be seen by the naked eye. Microscopy is the science of investigating small objects and structures which it is means invisible to the eye unless aided by a microscope such as microorganisms (bacteria, fungi, and parasite) & other cells. Antony Van Leeuwenhoek (1632-1723) invented microscope, which it in 1674 built a simple microscope with only one lens to examine blood, yeast, insects and many other tiny objects. There are many types of microscopes, and they may be grouped according to way the instruments interact with a sample to create images, either by sending a beam of light or electrons to a sample in its optical path, or by scanning across, and a short distance from the surface of a sample using a probe. 2 Magnification of microscope:

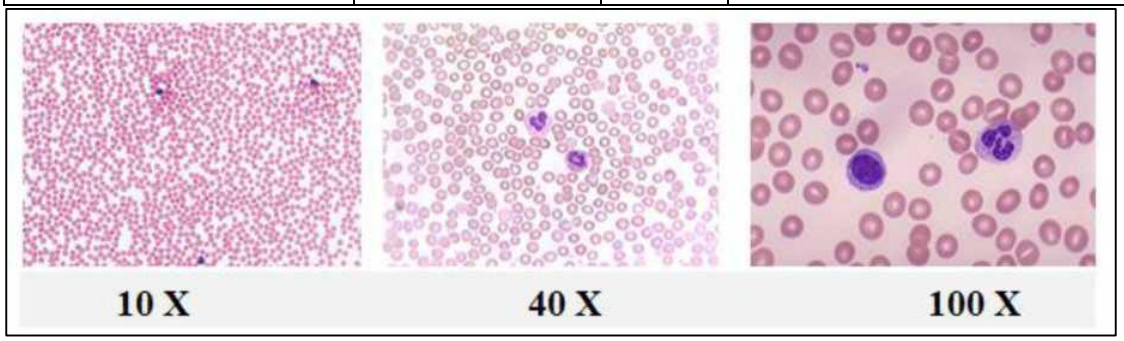
***Magnification**: defined as the degree of enlargement of the image of the object achieved by the microscope, the magnifying power consists from the eyepieces and objectives lens power.

***The working** distance is the distance between the objective lens and the specimen on the stage. The higher the magnifying power of the objective the shorter is the working distance.



the total magnification achieved in the below table:

Objective lens	Ocular lens	Oil	Magnification
4 x	10 x	No	4 X 10 = 40 times
10 X	10 x	No	10 X 10 = 100 times
40 X	10 x	No	40 X 10 = 400 times
100 X	10 x	Yes	100 X 10 =1000 times



<u>Resolution of microscope</u>

ability of a lens to separate or distinguish small objects that are close

together. It is considered the wavelength of light used is major factor in resolution (shorter wavelength = greater resolutions it is considered the resolutionis power to show details clearly. Both the **Magnification &Resolution** are needed to see a clear image

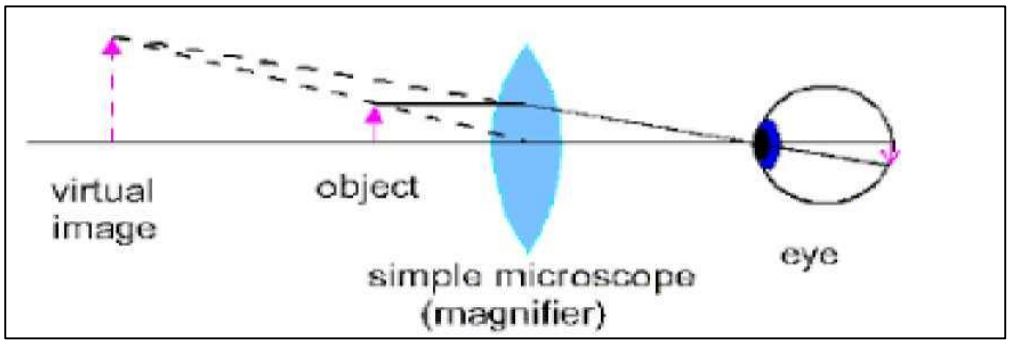
Types of Microscopes:

The lens system classification divides the microscope into **simple** or **compound** microscopes

A- Simple microscope consists of a single lens or several lenses grouped in one unit and are only used to **enlarge** an object.The lens system ranges from **double convex** to **two plano-convex** lenses. Examples of simple microscopes include reading glasses, jewelry eyepieces.

Principle Of Simple Microscope

A simple microscope is used to obtain small magnifications. It is usually to used for study of microscopic algae, fungi and biological specimen. ٭ Light from a light source (mirror) passes through a thin transparent object. A biconvex lens magnifies the size of the object to get an enlarged virtual image.



Parts of a Simple Microscope:

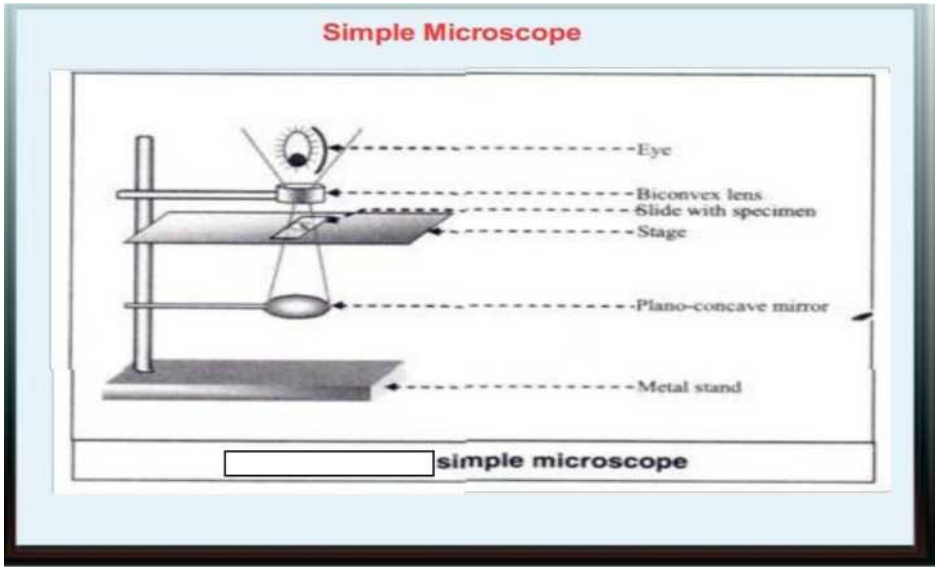
1) Mechanical parts 2) Optical parts

1- **Mechanical parts:** These parts support the optical parts and help in their adjustment for focusing the object ,They include the following components^**Met Stand, Stage**

2- **Optical parts:** These parts are involved in passing the light through the object (specimen) and magnifying its size .The components of the optical parts are as follows:

- **Mirror**: **A Plano-convex** mirror is fitted below the stage to the vertical rod by means of a frame. It focuses the surrounding light on the object to be observed.

- **Lens**: **A biconvex lens** is fitted above.the stage, to the vertical rod, by means of a frame. It magnifies the size of the object and the enlarged virtual image formed is observed by keeping the eye above it. For proper focusing, the lens can be moved up and down by the frame.



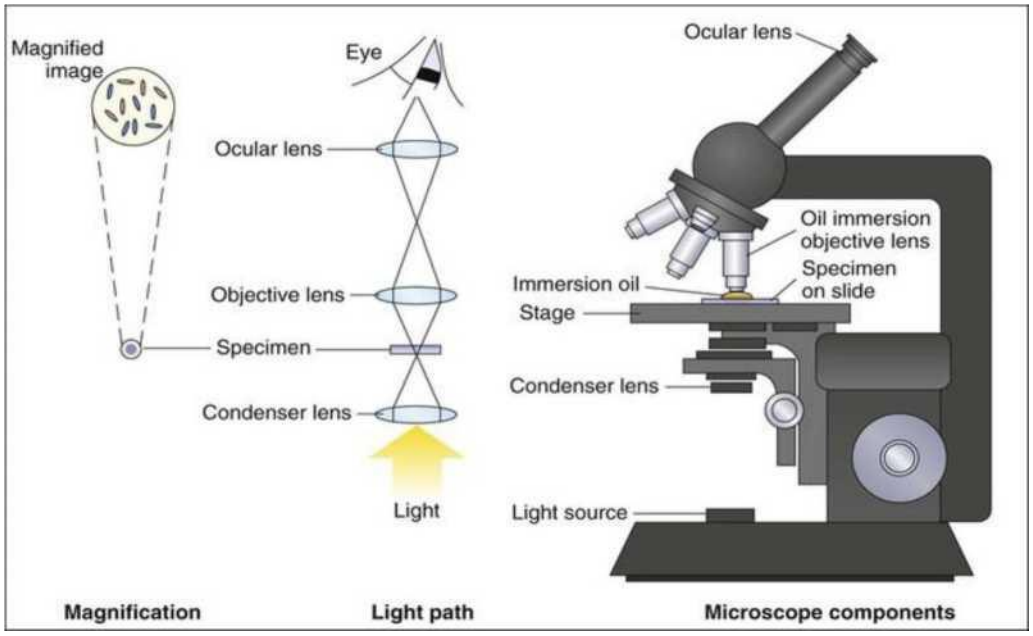
B- compound microscope includes an **eyepiece** and one or more **objectives** where the eyepiece enlarges the resolved image created by the objective. the are many type of compound microscope:

!-Brightfield microscopy is the most elementary form of microscope illumination techniques and is generally used with compound microscopes. "brightfield" is derived from the fact that the specimen is dark and contrasted by the surrounding bright viewing field. Simple light microscopes are sometimes referred to as bright field microscopes.

Principle of the bright-field microscopy :

In bright-field microscopy a specimen is placed on the stage of the microscope and the microscope's light source is aimed at a lens beneath the specimen , This lens is called a condenser. The condenser usually contains an aperture diaphragm to control and focus light on the specimen; light passes through the specimen and the is collected by an objective lens situated in a turret above the stage.

in transmitting light microscopes the **illumination system** is very important. The system includes the **light source, condenser**, and **iris**. A **condenser** is usually a combination of lenses that gathers and concentrates light in a specified direction, under the stage. The **iris** controls the intensity of the light that goes into the condenser. The **light source** is a variety of bulbs that create just the right kind of light with little heat.



Difference Between Simple And Compound Microscope

Characteristics	Simple Microscope	Compound Microscope
Number of lenses	One to magnify objects	3-5 to magnify objects
Condenser lens	Absent	Present
Light source	Natural	Illuminator
Mirror type	Concave reflecting	One side is plain and the other side is concave
Magnifying power	Up to 300X	2,00OX
Adjusting Magnification	No	Yes

Used	At basic level	By professional for research purpose

The electron microscope:

Is a type of microscope that uses electrons to illuminate a specimen and create an enlarged image. Electron microscopes have much greater resolving power and higher magnifications. Some electron microscopes can magnify specimens up to 2 million times. The greater resolution and magnification of the electron microscope is due to the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, so can reveal the structure of smaller objects .

** Components of Electron Microscope:

1. Electron gun

2. Electromagnetic lensesthree sets.

3. Image viewing and recording system. Electron gun is a heated tungsten filament, which generates electrons. Condenser lens focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam.

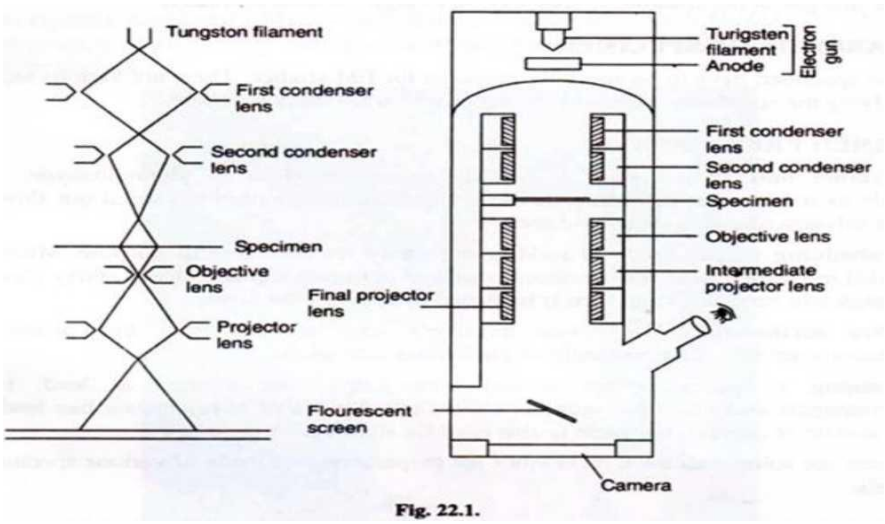


Fig. 22.1.

**There are two main types of electron microscope:

1- The transmission electron microscope (TEM) : TEM forms image when radiations pass and are transmitted through the specimen, is used to view thin specimens or for viewing internal features that are inside or beyond the surface (e.g. organelles, macromolecules, atoms).

2-The scanning EM (SEM): SEM produces images by detecting secondary electrons which are emitted from the surface of the specimen due to excitation by the primary electron beam. Therefore SEM is used to examine the surfaces of the microorganisms in great detail. like study the atomic composition of specimens, example, the surface distribution of immune-labels.

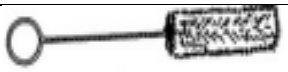
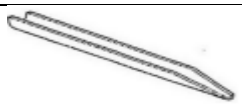
المحاضرة الثانية

Laboratory Equipment and Functions

Name	Picture	Use
Thermometer		Used to measure temperature
Pipet		Used to measure and dispense small amounts of liquids (ex. 1 mL)
Well plate		Mixing very small amounts of chemicals together and comparing results.
Corks		Used to seal or stop flasks or test tubes.
Hot plate		used to heat substances that may be flammable.
Florence flask		Flask with a round body and flat bottom. Used to hold and heat liquids.

Graduated cylinder		Measuring specific amounts of liquids (65 mL)
Spatula		Measuring/removing small amounts of solids or powders (often when obtaining mass)
Wash bottle		Used to wash down specific pieces of equipment with water or keep materials moist.
Micropipets		Used to measure and dispence very small amounts of liquids. (ex. 0.5 mL)
Burette		Measuring specific amounts of liquids (often determining amounts of acids or bases needed) (ex. 13 mL) .
Dropper		Used to obtain small amounts of liquids (not precise)

Iron ring		Supports a beaker over a bunsen burner. Wire gauze is usually placed on top of this structure.
Utility clamp		Used to hold a test tube or other piece of equipment in place on a ring stand.
Wire gauze		Suspending glassware over the Bunsen burner
Tongs		Transport a hot beaker; remove lid from crucible.
Triple-beam balance		Obtaining the mass of an object
Test tube clamp		Heating contents in a test tube

Bunsen burner		Heating (flame-safe) contents in the lab
Forceps		Used in dissection to grasp tissues or pick up small items.
File		Used to grind down materials or sharpen items.
Wire brush		Used to clean the inside of test tubes or graduated cylinders
Test tube rack		Holding many test tubes filled with chemicals (or for drying after washing)
Funnel		Used to pour liquids into containers with small openings; also used to hold filter paper
Scoopula		Scooping solids/powders.

Ring stand		Supports the bunsen burner, iron ring, pipestem triangle, and other items, often while heating a substance.
Evaporating dish		Used to evaporate excess solvents to create a more concentrated solution.
Test tubes		Holds small amounts of liquids for mixing or heating.
Beaker		Holding water (also used to heat liquids)
Erlenmeyer flask		Narrow-mouthed container used to transport, heat, or store substance. Often used when a stopper is required.

Volumetric flask		Flask calibrated to contain a precise volume at a particular temperature. Used for precise dilutions and creating standard solutions.
Watch glass		Keeping liquid contents in a beaker from splattering
Mortar & pestle		Used to grind chemicals to powder
Petri dishes		A cylindrical container made of plastic and used to culture cells, fungi and bacteria
Lab coats		Long shirt worn by medical professionals to protect against chemicals and pollutants
Safety goggles		It is used to protect the eyes from flying particles, water or chemicals

gloves		Used to protect hands from chemicals

المحاضرة الثالثة

Centrifuge

It is an equipment that makes object revolve around in a fixed axis (spins it in a circle) by applying centrifugal force to the axis of spin at a different speed for separation of two immiscible material. Generally, comprise a rotor mainly made of aluminium is very compact and it contains two, four, six, or more numbered wells are using for put the tubes of sample.

Principles of centrifugation

Centrifugation is a technique used for the separation of particles using a centrifugal field. The particles are suspended in liquid medium and placed in a centrifuge tube. The tube is then placed in a rotor and spun at a definitive speed. Rotation of the rotor about a central axis generates a centrifugal force upon the particles in the suspension.

Two forces counteract the centrifugal force acting on the suspended particles:

- Buoyant force: This is the force with which the particles must displace the liquid media into which they sediment.

- Frictional force: This is the force generated by the particles as they migrate through the solution.

Particles move away from the axis of rotation in a centrifugal field only when the centrifugal force exceeds the counteracting buoyant and frictional forces resulting in sedimentation of the particles at a constant rate.Particles which differ in density, size or shape sediment at different rates.

The rate of sedimentation depends upon:

1. The applied centrifugal field
2. Density and radius of the particle.
3. Density and viscosity of the suspending medium

Type of centrifuge:

1- **General-purpose centrifuges**: can be using for many applications, which include a variety of rotors and speed (up to 5000 rpm), common in clinical specimens such :blood (plasma/serum separation) and urine sample.

Test tube: for isolation, the serum from blood & other sample or material, may be made of plastic or glassware with color top.

EDTA tube: for isolation, the plasma form blood.

Gel tube: It has no anticoagulant, usingfor isolation the serum form whole blood Note/ for separation blood put the tube in the centrifuge for 5–10 min at 3000-5000 rpm

General centrifuges

2- **Micro centrifuges:** are used to small volumes separation such biological molecules, cells, or nucleus. Micro centrifuge tubes generally hold 0.5 - 2.0 mL of liquid, by angular speeds of 12,000–15,000 rpm.

3- **Refrigerated centrifuges:** for samples, that requiring cool temperatures such DNA, RNA, protein &mitochondrial. This centrifuge should be able to run at its maximum speed and still maintain a consistent temperature. Speed range up to 15,000 rpm.

4- **Haematocrit centrifuges** are using to measure the volume percentage of red blood cells in whole blood.

Hematocrit centrifuges:

Devices used to determine the blood's hematocrit—the ratio of red blood cell volume to whole blood volume, expressed as a percentage. By put the blood in wintrobe tube or capillary tube and put in hematocrit centrifuges.

Haematocrit centrifuges

Wintrobe tube or capillary tube

Parts of hematocrit centrifuge:

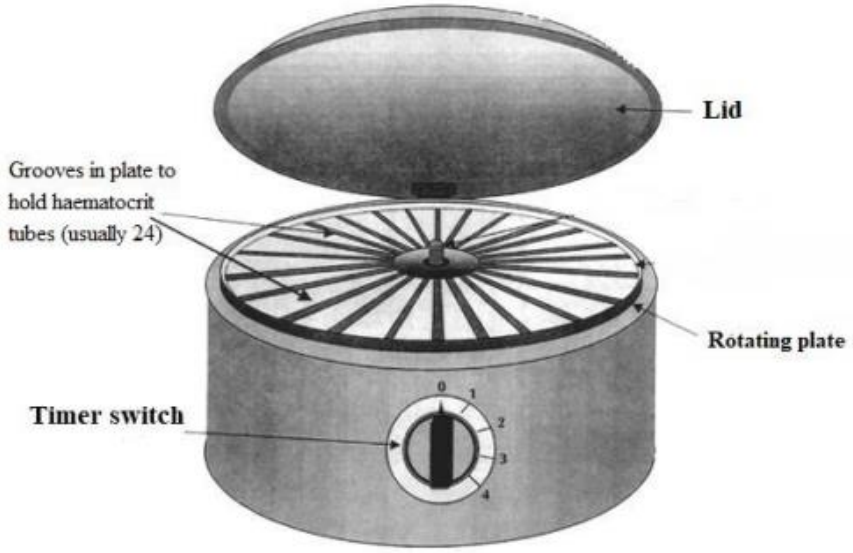
1) Lid

2) Rotor (rotating plate).

3) Centrifuge heads (carriers): that spins on the rotor.

4) Grooves in plate to hold haematocrit tubes.

5) The centrifuge head contains the cups that cover the rotor.



Haematocrit centrifuges parts

Balances

are essential laboratory instruments that are widely used for weighing of various substances (powders, crystals and others) in the laboratory. For instance, to prepare reagents, stains and culture media, balances are required to weigh accurately and precisely within the needed range.

Types of balances in medical laboratory may be:

a) Rough balances (mechanical balances).

b) Analytical balances (Sensitive balance).

a) Rough balances:

Rough balances are mechanical balances found in several types, some of them comprise is sliding scale and some have a single or double pan. While it operating, not require mains electricity or battery power and less expensive than analytical balances.

It is using in:

1. To weigh large amounts (gram to several kilograms).

2. When a high degree of accuracy is not required.

b) Analytical balances (Sensitive balance):

Sensitive balances are electronic balances that comprise of single pan balances that use an electron magnetic force instead of weights and found in inside a glass case. It requires mains electricity or battery power. Sensitive balances are a highly sensitive instrument that most used balances in medical laboratories to provide a precision and accuracy for reagent and standard preparation.

It is using in:

1. To weigh small quantities usually in gram (gm) to mili gram (mg) range.

2. When great accuracy is required.

Analytical balances

Operating and care of Sensitive balances

1. Always handle a balance with care.

2. Position the balance on a bench away from vibration and direct sunlight.

3. Before starting to weigh, zero the balance as directed by press the TARE or ZERO.

4. Put the container (dish, a beaker, a piece of folded paper or filter paper) and weigh the container first.

5. Press the TARE or ZERO button to get a reading of 0.00 g.

6. Put the material, and weigh the material at room temperature in a weighing the container. Never put the material directly on the balance pan.

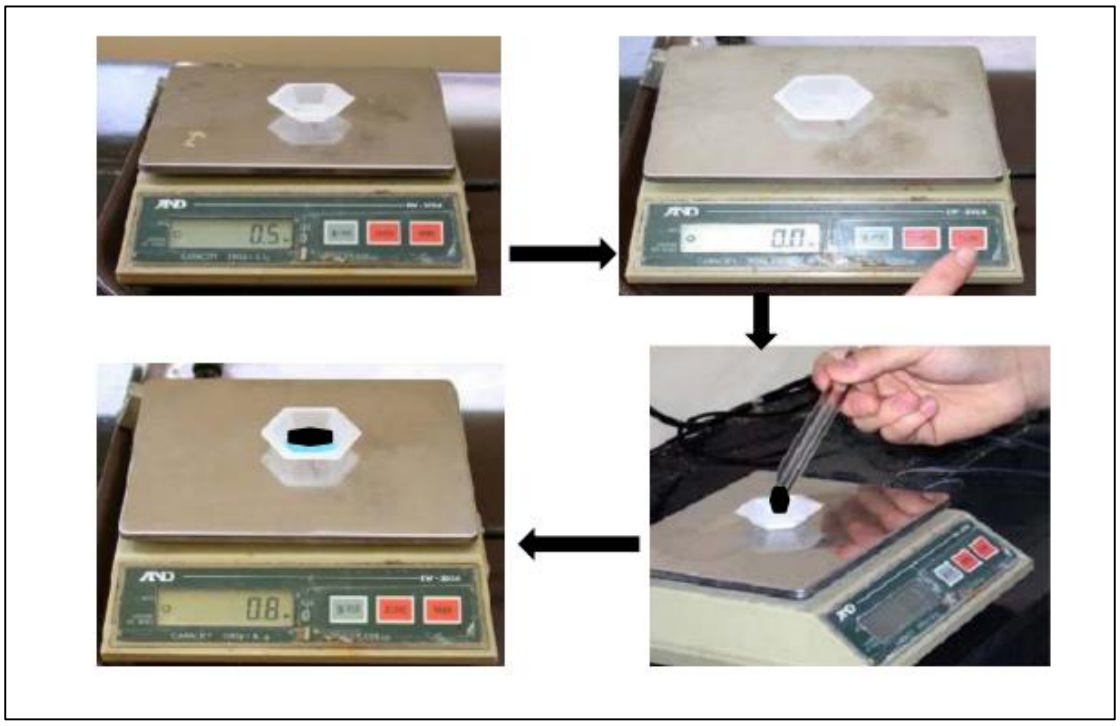
7.When you're finished with the balance, return the weights back to zero. Then it will be ready for the next person to use it.

8. Always use forceps to add or remove the material.

9. Protect the balances from dust, moisture and fungal growth.

10. Use small brush to remove any chemical, which may have been spilt on the balance.

11. Keep the balance is clean.



المحاضرة الرابعة

Sterilization

Sterilization: defined as the destruction or removal of all microorganisms (virus, bacteria, fungus) and their spores by applying heat, irradiation, and filtration.

Disinfection: is the destruction of many microorganisms but not usually the bacterial spores such a chemical agents & detergent.

Sterilization methods

- **Sterilization by heat**
- **Sterilization by steam**
- **Sterilization by dry heat**
- **Sterilization by radiation**
- **Chemical sterilization**

Sterilization by saturated steam with high pressure using (autoclave)

Dry heat (oven) is the most common method available for ease of use.

Autoclave:

The autoclave is a pressure metal chamber provides the moist heat used to sterilization by applying a high pressure and high

temperature steam. A basic autoclave is similar to a pressure cooker is a device for heating the water above 100oC under pressure. Autoclave can sterilize anything that can withstand a temperature of 121oC for 15 minutes.

- ✓ French merchant Charles Chamberlain invented this device in 1879.
- ✓ The word autoclave is composed of the two Greek words; Namely, automatic and key-lock in Latin.

Uses Autoclave

An autoclave is used in medical applications to sterilize: surgical tools, laboratory tools, culture media and other materials. It can sterilize solids &liquids material. Use the power of steam to kill bacteria, spores and germs resistant to boiling water and detergents. Also using for sterilize the medical waste. The autoclave uses in industrial applications such vulcanization of rubber &the aerospace industry.

Disadvantages of sterilization by autoclave

- A device (steam sterilizer) that requires expertise to maintain to keep it in working condition.
- Requires strict adherence to time, temperature and pressure.
- Repeated sterilization cycles can damage the cutting edges of the machines (eg the scissors(.
- It cannot be used to sterilize plastic materials that cannot withstand temperatures high.

Hot air ovens:

Hot air ovens are electrical devices, which use dry high temperatures for many times to sterilize. The ovens use conduction to sterilize items by heating the outside surfaces of the item, which then absorbs the heat and moves it towards the center of the item.

- Generally, they can be operated from 50 to 300 °C, contain a thermostat for control on the temperature.
- Their double walled insulation keeps the heat in and conserves energy.
- The commonly-used temperatures and time that hot air ovens need to sterilize materials is:
  -160 °C for 60 minutes.
  -170 °C for 30 minutes.

Advantages of sterilization by hot air ovens

- Dry heat reaches all tool surfaces, including machines that cannot be used disassembled.
- Protect sharp objects or tools with a cutting edge.
- Leaves no chemical residue.
- Eliminates "wet pack" problems in humid climates.

Disadvantages of hot air oven sterilization

- Materials made of plastic and rubber cannot be sterilized by dry heat Because the temperatures used (160-170°C) are too high for these Materials.
- Dry heat penetrates the material slowly and unevenly.

Flame sterilization

In this method the material is passed over a Bunsen flame, but not heated to redness. Materials such as lancets, nozzles of test tubes, flasks, and slides are passed the glass over the flame several times. This method kills vegetative cells, there is not This ensures that germs are eliminated as a result of this short exposure. This style It is also limited to those materials that can be exposed to flame. Cracking may occur Glassware.

Radiosterilization

Many types of radiation are used for sterilization, such as

- electromagnetic radiation (such as gamma rays , X-rays and ultraviolet)
- particulate radiation such as (beam-E.)

Ultraviolet light irradiation(UV)

- Ultraviolet rays have been used to help disinfect the air for more than 50 years , UV rays stop infection in the air in closed environments.
- Ultraviolet rays can only kill living organisms minutes directly exposed to x-ray light ultraviolet. Surfaces are not accessible her by ultraviolet radiation

Gamma radiation sterilization

- It is usually used to sterilize medicines In final packaging an sterilization of gas, liquids and solids and disposable cosmetics and gloves Urinalysis tubes and test tubes. and medical equipment, such as syringes needles

rays-X radiation sterilization

- X-rays are a form of ionizing energy and they can sterilize Medical devices. It is an electricity based process and it does not require any electricity chemicals or radioactive materials. At present, x-rays are not An official method for sterilizing medicines and medical devices.

E-beam sterilization

- The beam-E sterilization method has recently attracted more attention for device sterilization Medical and has many advantages such as being safe, no emissions and high processing speed Exposure time is short

Chemical sterilization

Chemical sterilization is used to sterilize and sterilize objects that have been damaged by steam, high pressure, dry heat sterilization, or sterilization. In the case of the device, sterilization is not available.

Ethylene oxide (EtO) sterilization

- ❖ Use ethylene oxide (EO) as a low temperature sterilizer in the field Health care since 1950. It is a colorless, odorless, and flammable gas.
- ❖ It is used to sterilize sensitive surgical instruments For heat and moisture, such as plastics and precision instruments.

Gas plasma sterilization (hydrogen peroxide based)

- ❖ Gas sterilizes at a low temperature for many heat-sensitive medical devices and humidity
- ❖ The sterilization cycle takes between 45-55 minutes.
- ❖ Does not penetrate well, and cannot be used on paper or linen.