# Medical Laboratory Techniques and Quality Control

Technical Institute of Babylon
Medical Laboratory Department
First class

**Assistant Prof. Dr. Hayder hamzah Ibrahim**

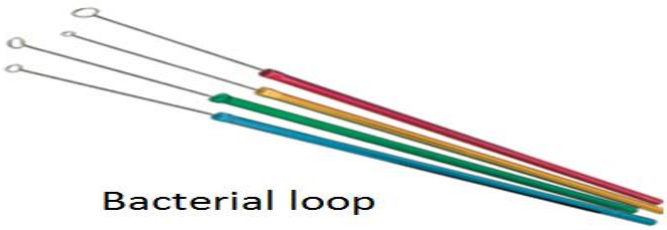
# Laboratory Room

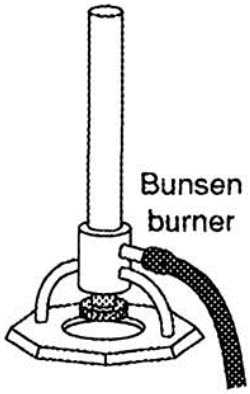
# 1

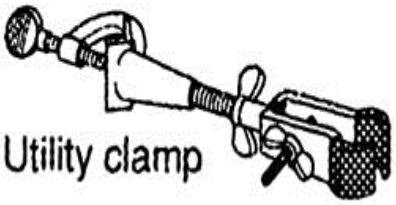
Medical Laboratory

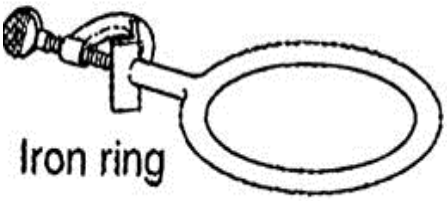
**Laboratory** is defined as a room for the testing, analysis and examination of articles**.**

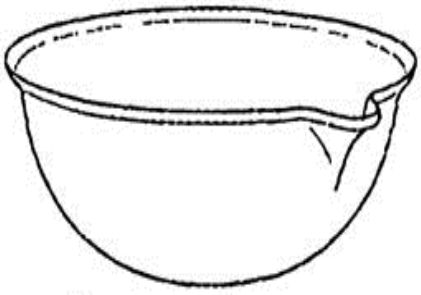
**Laboratory instruments**


Bacterial loop





Utility clamp

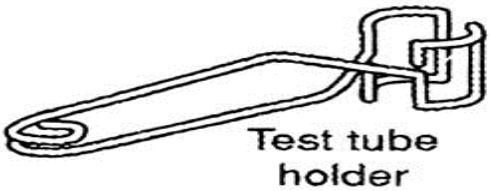

Iron ring

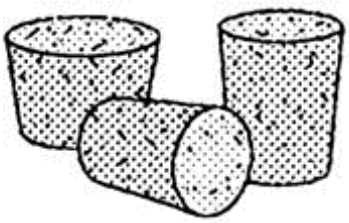

Evaporating dish

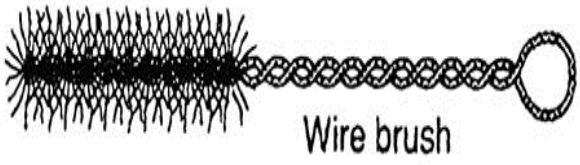

Mortar and
pestle


Crucible
and cover


Crucible
and cover

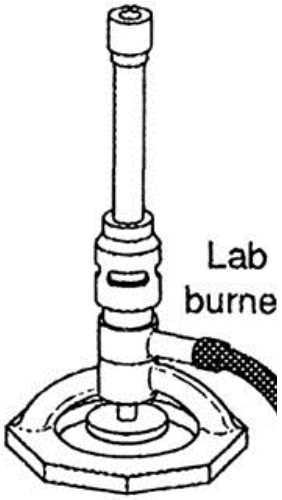

Test tube
holder


Corks

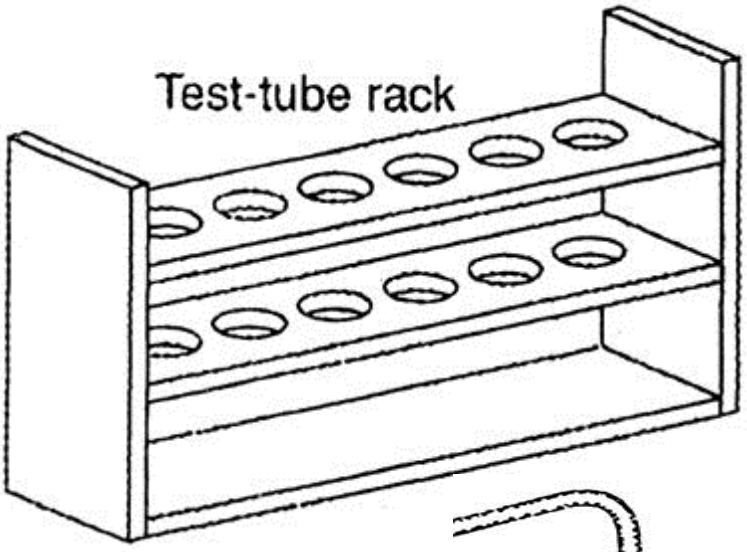


Wire brush

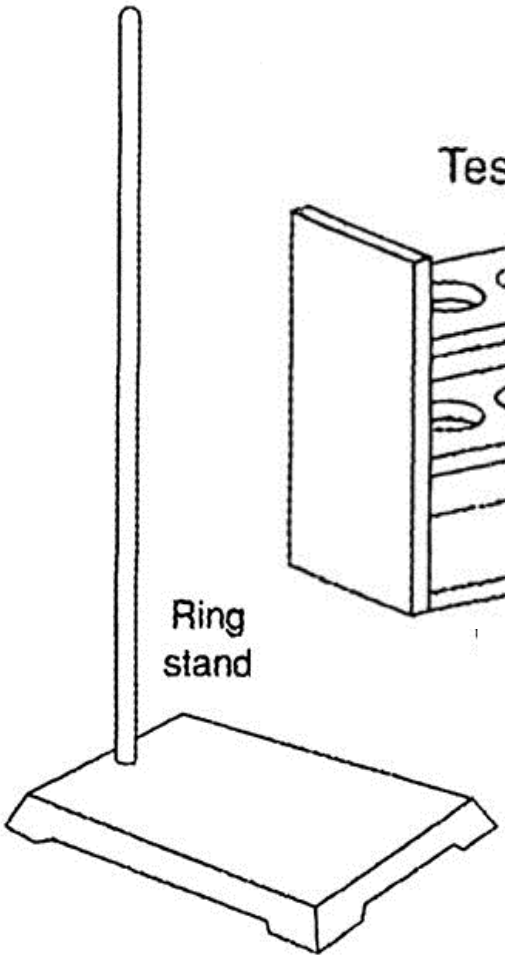
Forceps



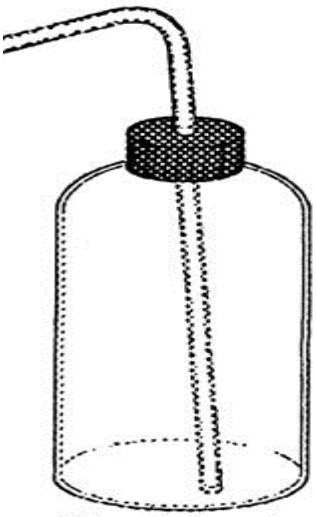
Lab burne



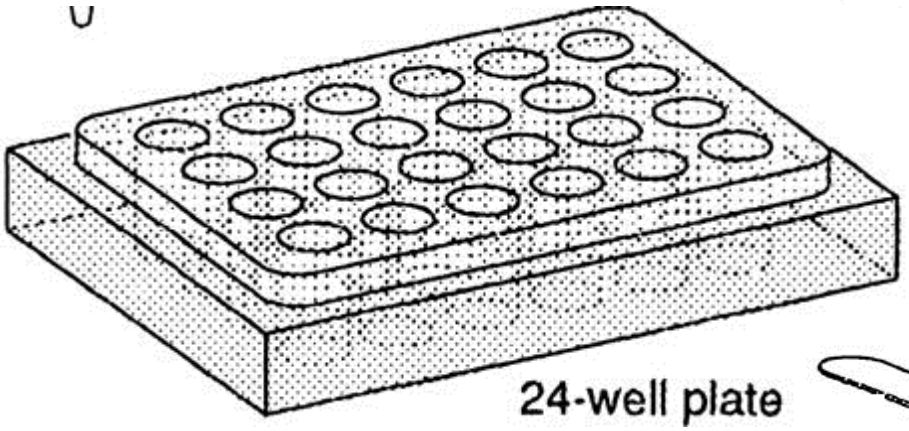
Test-tube rack



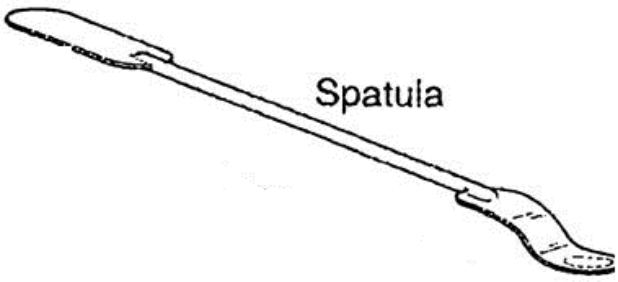
Ring stand



Wash bottle

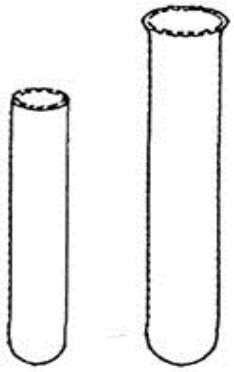


24-well plate

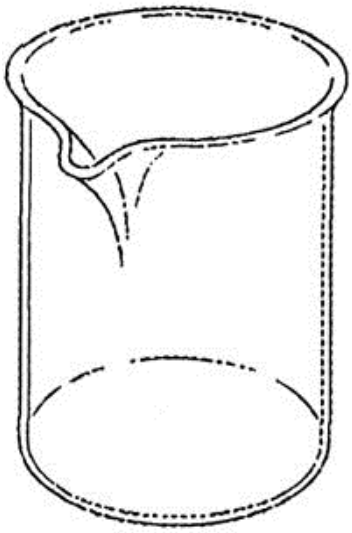


Spatula

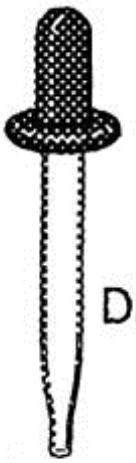
Laboratory glasses



Test tubes



Beaker

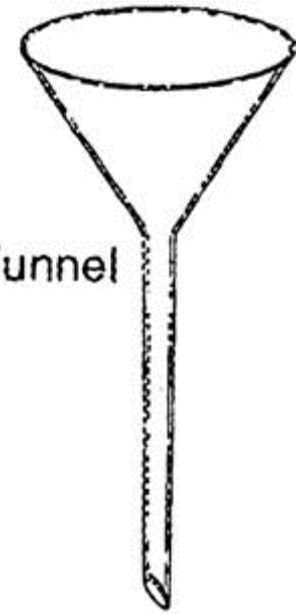


Dropp

Watch glass

Pipet

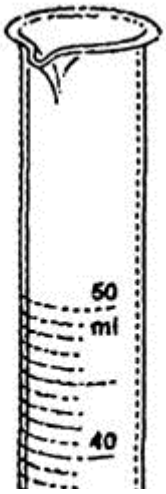
Thermometer



Funnel

Glass rod


volimetric flask




Graduated cylinder

Petri dish

Laborat

y

## What are the precautionary measures inside the laboratory room1?


**1. Listen to or read instructions carefully before attempting to do anything.**
**2. Wear safety goggles to protect your eyes from chemicals, heated materials, or things that might be able to shatter.**
**3. Notify your teacher if any spills or accidents occur.**
**4. After handling chemicals, always wash your hands with soap and water.**
**5. During lab work, keep your hands away from your face.**
**6. Tie back long hair.**
**7. Roll up loose sleeves.**
**8. Know the location of the fire extinguisher, fire blanket, eyewash station, and first aid kit.**
**9. Keep your work area uncluttered. Take to the lab station only what is necessary.**
**10. It is suggested that you wear glasses rather than contact lenses.**
**11. Never put anything into your mouth during a lab experiment.**
**12. Clean up your lab area at the conclusion of the laboratory period.**
**13-No eating or drinking .**

**Safety signs:-** Are a colours and symbols appropriately used can provide ever-present information and warnings of hazards which are essential to safety at work, and independent of language**.**



+ eye protection

+ head protection

+ ear protection

+ hand protection

+ respiratory system protection

+ foot protection

Compressed Gas

Flammable

Oxidizer

Poisonous

Toxic

Biohazard

Corrosive

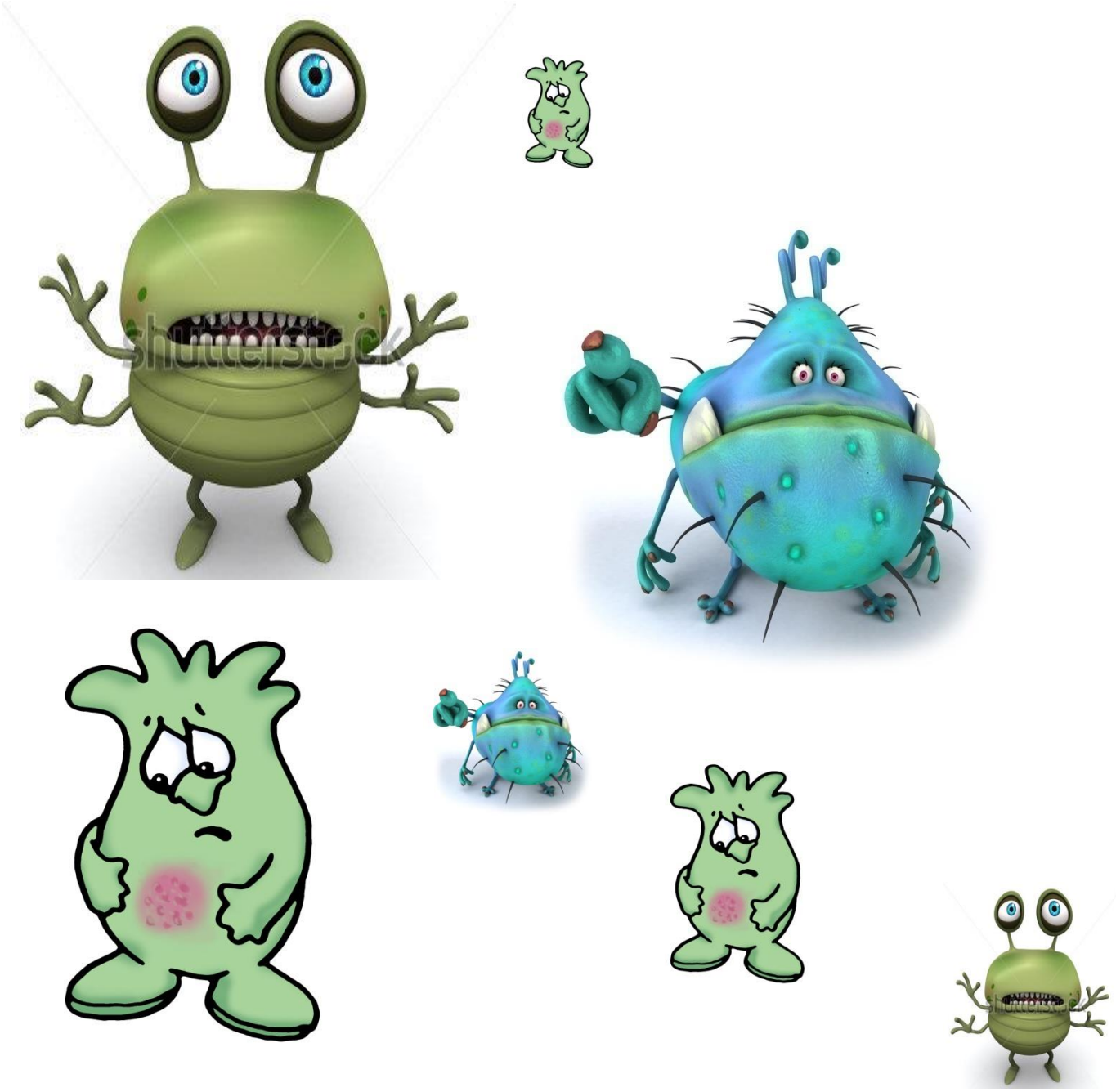
Reactive


NO FOOD OR DRINK

IN LAB

# Microbiology

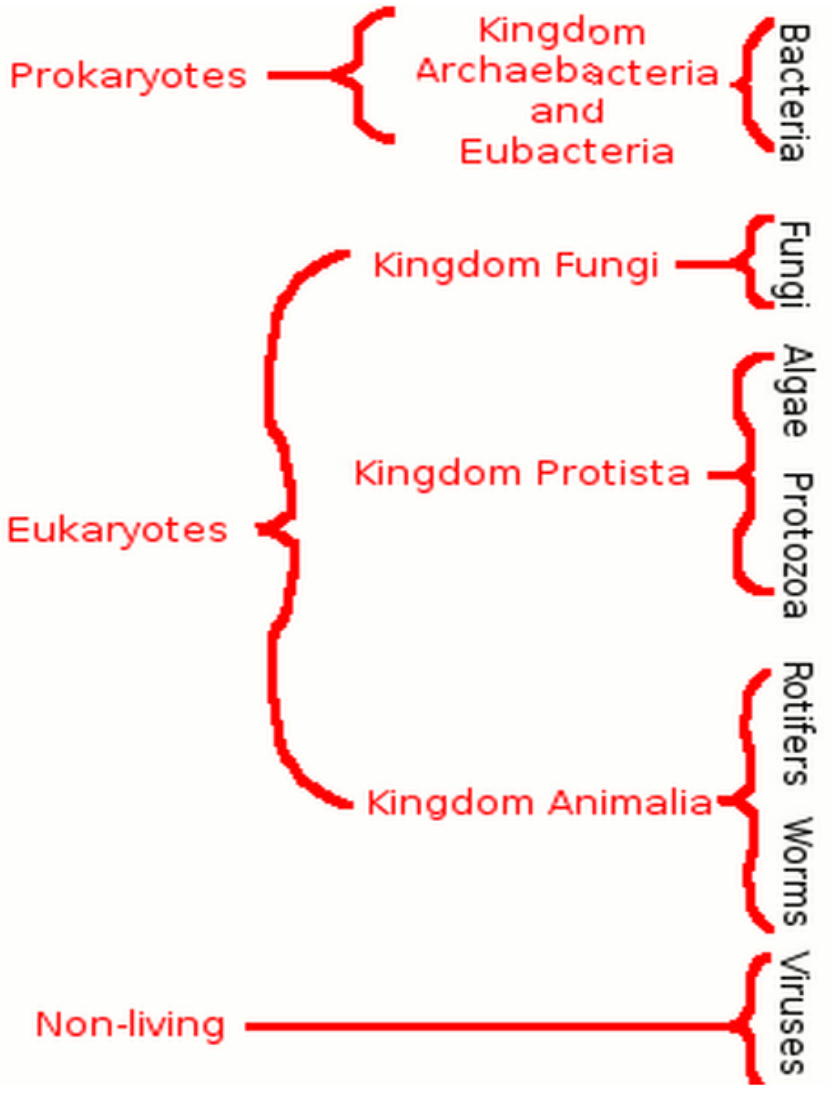
## 2



All living organisms can be sorted into one of two groups depending on the fundamental structure of their cells. These two groups are the **prokaryotes** and the **eukaryotes**.

**Prokaryotes** are organisms made up of cells that lack a cell nucleus or any membrane-encased organelles.

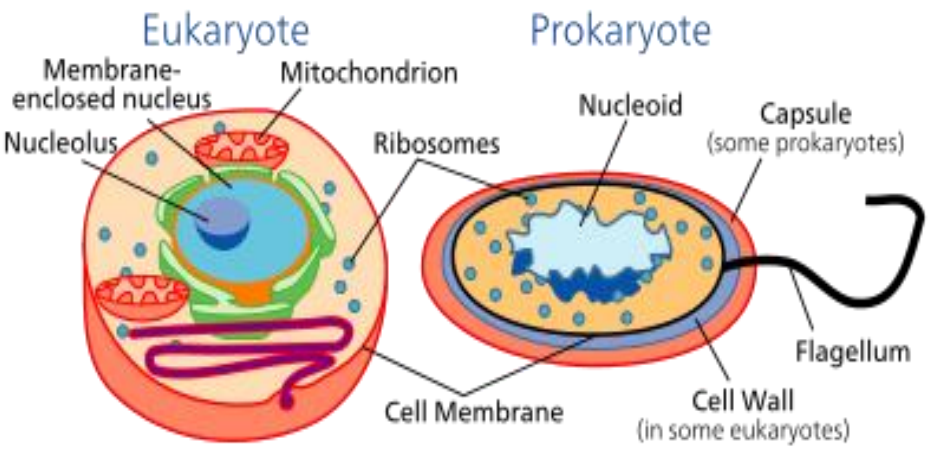
**Eukaryotes** are organisms made up of cells that possess a membrane-bound nucleus (that holds genetic material) as well as membrane-bound organelles.




## Microorganism classification

**Compare between eukaryotic and prokaryotic cells**

| Characteristics | Prokaryotes | Eukaryotes |
|---|---|---|
| **Size of cell** | **Typically 0.2-2.0 mm in diameter** | **Typically 10-100 mm in diameter** |
| **Nucleus** | **No nuclear** | **True nucleus** |
| **organelles** | **Absent** | **Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria and chloroplasts** |
| **Flagella** | **Consist of two protein building blocks** | **Complex; consist of multiple microtubules** |
| **Cell wall** | **Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)** | **When present, chemically simple** |
| **Plasma membrane** | **Absent** | **Present** |
| **Cytoplasm** | **No cytoskeleton or cytoplasmic streaming** | **Cytoskeleton; cytoplasmic streaming** |
| **Ribosomes** | **Smaller size (70S)** | **Larger size (80S); smaller size (70S) in organelles** |
| **Chromosome (DNA) arrangement** | **Single circular chromosome** | **Multiple linear chromosomes** |
| **Cell division** | **Binary fission** | **Mitosis** crdf |
| **Sexual reproduction** | **No meiosis; transfer of DNA fragments only (conjugation)** | **Involves meiosis** |

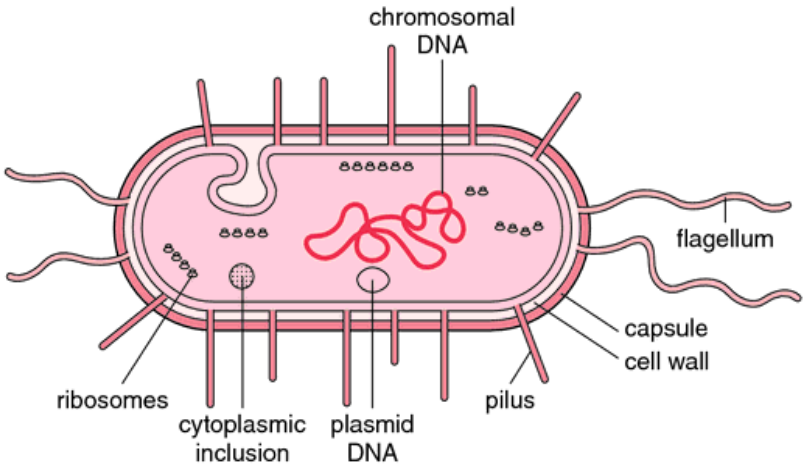


## Compare between eukaryotic and prokaryotic cells

**Microbiology:** - is the study of microscopic organisms, either unicellular (Bacteria), multicellular (fungi), or acellular (virus)**.**

**Note :-** Viruses are not living organisms. They lack the enzymes necessary for the production of energy, proteins, and nucleic acid. To replicate, viruses must invade host cells.

**Bacteria: - Is** single-celled microorganisms that can exist either as independent (free-living) organisms or as parasites (dependent on another




organism for life).

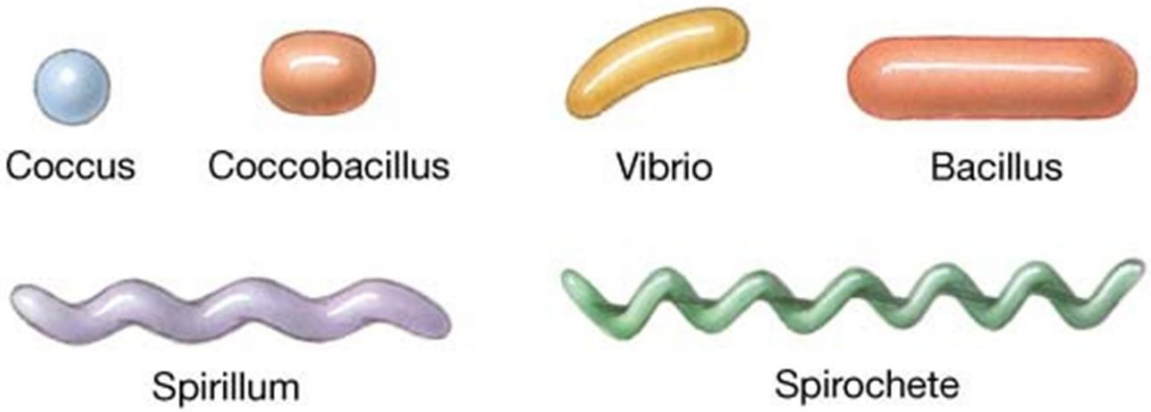
# Structures of bacteria cell

**Summary of characteristics of typical bacterial cell structures**

| Structure | Function(s) | Predominant chemical composition |
|---|---|---|
| Flagella | Swimming movement | Protein |
| Pili | | |
| Sex pilus | Stabilizes mating bacteria during DNA transfer by conjugation | Protein |
| Common pili or fimbriae | Attachment to surfaces; protection against phagotrophic engulfment | Protein |
| Capsules (includes "slime layers" and glycocalyx) | Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; reserve of nutrients or protection against desiccation | Usually polysaccharide; occasionally polypeptide |
| Cell wall | | |
| Gram-positive bacteria | Prevents osmotic lysis of cell protoplast and confers rigidity and shape on cells | Peptidoglycan (murein) complexed with teichoic acids |
| Gram-negative bacteria | Peptidoglycan prevents osmotic lysis and confers rigidity and shape; outer membrane is permeability barrier; associated LPS and proteins have various functions | Peptidoglycan (murein) surrounded by phospholipid protein-lipopolysaccharide "outer membrane" |
| Plasma membrane | Permeability barrier; transport of solutes; energy generation; location of numerous enzyme systems | Phospholipid and protein |
| Ribosomes | Sites of translation (protein synthesis) | RNA and protein |
| Inclusions | Often reserves of nutrients; | Highly variable; |

| | | |
|---|---|---|
| | additional specialized functions | carbohydrate, lipid, protein or inorganic |
| Chromosome | Genetic material of cell | DNA |
| Plasmid | Extrachromosomal genetic material | DNA |

**Bacterial forms there are different forms of bacteria include:-**

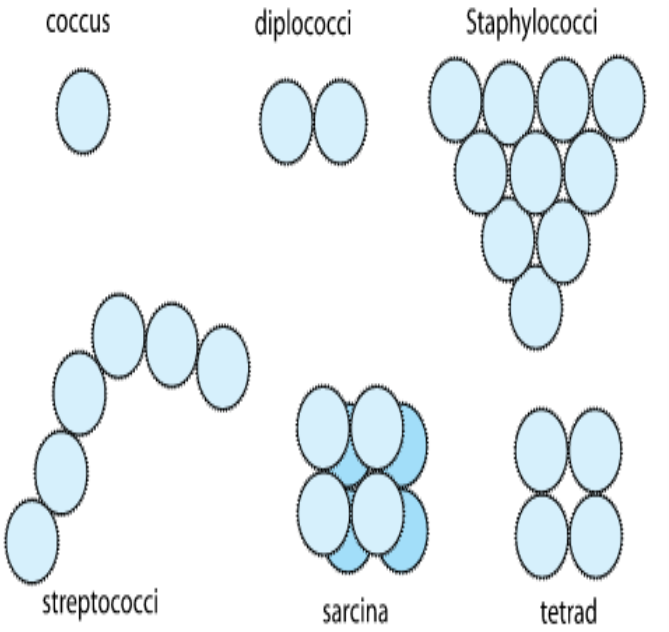


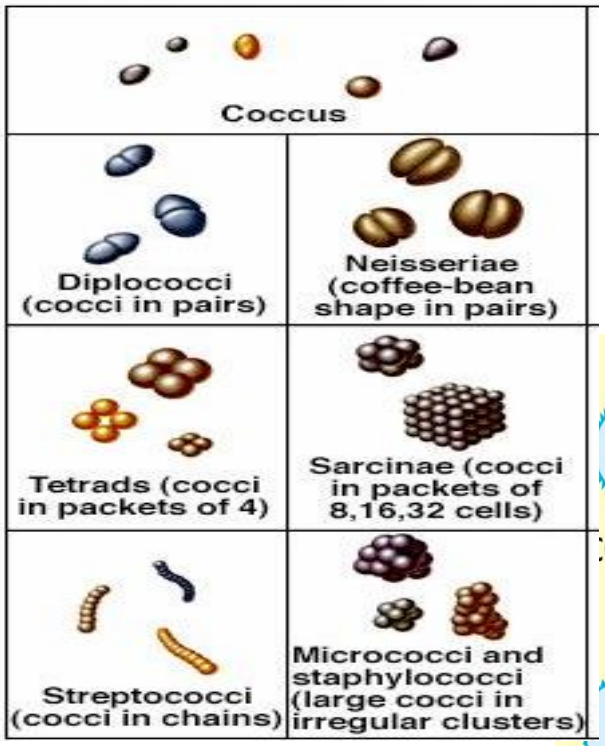

## Different bacterial forms

**Bacterial aggregation**

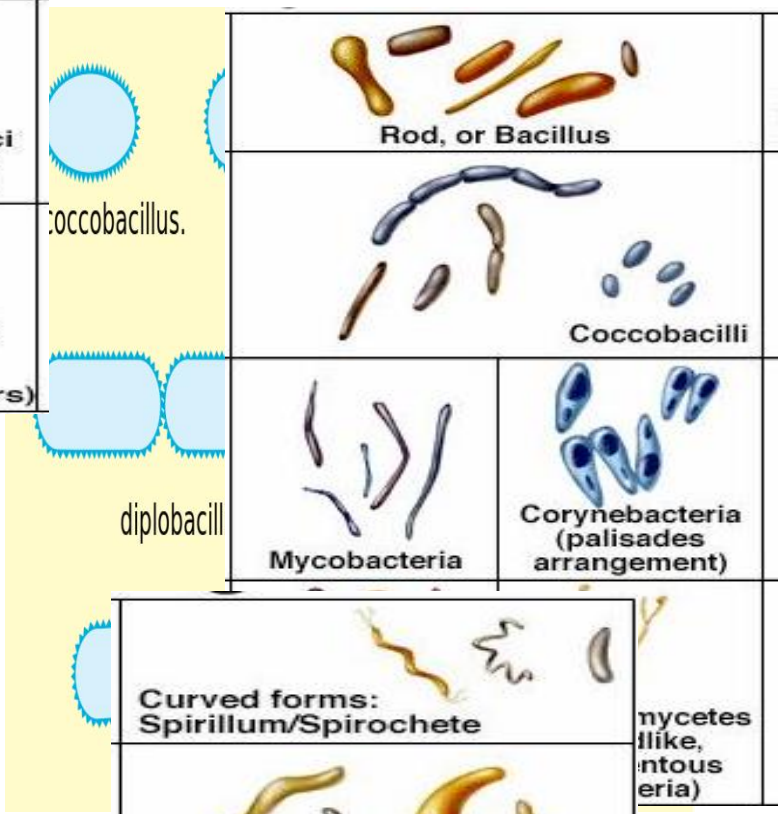
Bacteria are classified by direct examination with the light microscope through its morphology and aggregation.

1-Types of coccus bacterial aggregation.

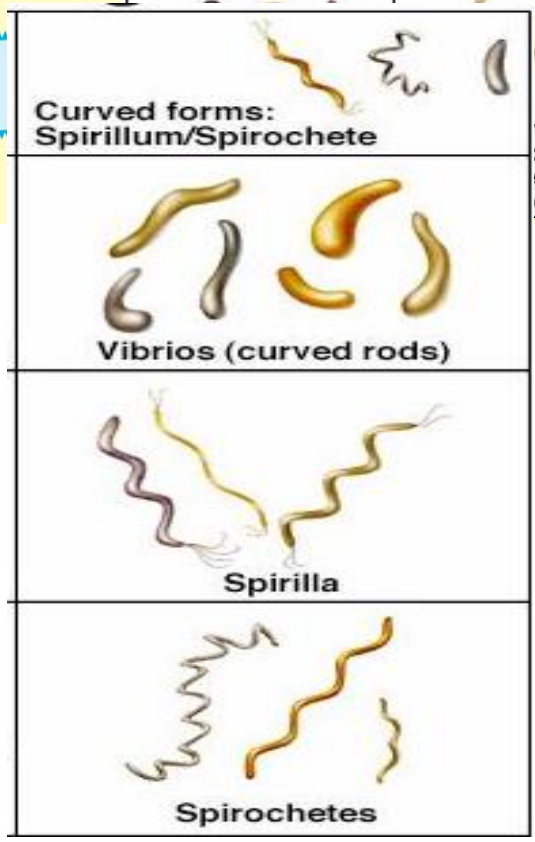





2-Types of bacillus bacterial aggregation.




3- Types of Spirochetes aggregation.



# Physiology of Bacteria

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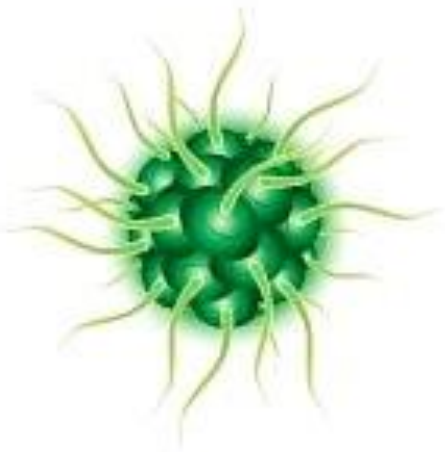

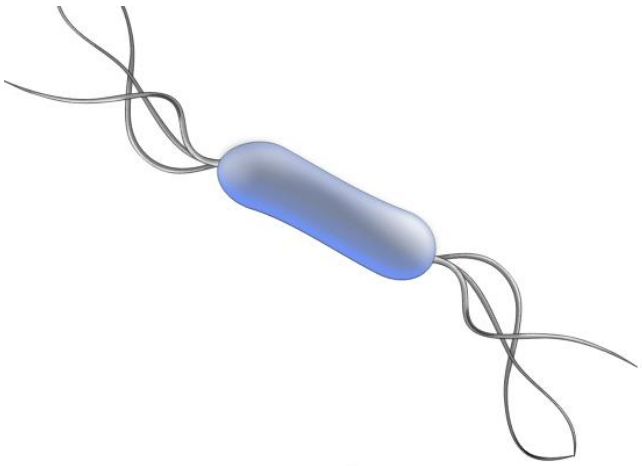

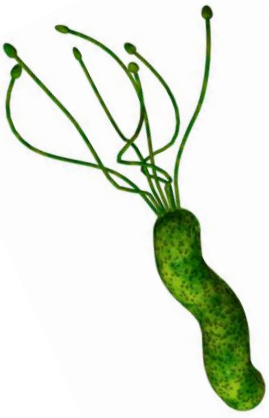

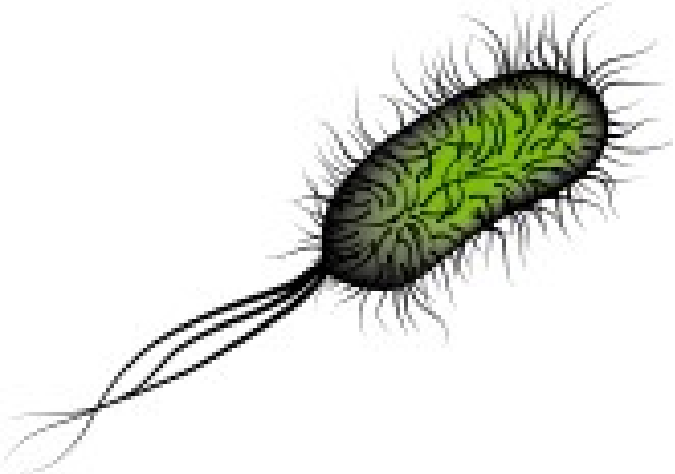

# Metabolism of Bacterial

**Microbial metabolism** is the means by which a microbe obtains the energy and nutrients (e.g. carbon) it needs to live and reproduce.

Bacteria must absorb molecules from the surrounding environment. Many macromolecules such as starch, proteins and lipids are too large to pass through the cell wall and membrane and, therefore, cannot be used directly as nutritional sources because the molecules. Many bacteria solve this problem by secreting enzymes called extracellular to the outside of the cell that degrade macromolecules into smaller compounds that can be taken up by the cells and further metabolized by intracellular enzymes. Examples of extracellular enzymes are amylase which degrades starch, protease for proteins, lipase for lipids, and DNase, which degrades DNA.

**There are two processes of metabolism**

**Anabolism:** Synthesis of more complex compounds and use of energy.

**Catabolism:** Break down substrate and release energy.

## Classification of microbial metabolisms

**1. According to obtain of carbon for synthesizing cell mass:**

**I-Autotrophic** – carbon is obtained from carbon dioxide ($CO_2$).

**II-Heterotrophic** – carbon is obtained from organic compounds.

**III-Mixotrophic** – carbon is obtained from both organic compounds and by fixing carbon dioxide.

**2. According to obtains of reducing equivalents used either in energy conservation or in biosynthetic reactions:**

**I-Lithotrophic** – reducing equivalents are obtained from inorganic compounds.

**II-Organotrophic** – reducing equivalents are obtained from organic compounds.

**3. According to of energy for living and growing:**

**I-Chemotrophic** – energy is obtained from external chemical compounds

**II-Phototrophic** – energy is obtained from light

## Classification of bacteria to different types:-

**1- Ecological classification :- the class divided into :-**

1-Indigenous:-Bacteria present in all time (intestine , soil , water, and present in all months of years).

2-Zymogenous :- That present in determined location.

3-Invaders :- these Bacteria common from other location.

**2-Oxygen requirement classification:- these classes classification according** to requirement bacteria to oxygen :-

1-Obliger aerobic bacteria: - need the $O_2$ when growth

2-Obliger anaerobic bacteria:- don't need $O_2$ when growth

3-Phoqulated (Facultative anaerobic) :-Growth when present or not present the $O_2$.

4-Microerophilic: - need the $O_2$ in small amount.

**3-Temperture of growth classification**

According to the optimum temperature for group bacteria classification into 4 groups.

1-Mesophilic bacteria :- Optimum temperature 25-35 °C and growth range temperature 15-45°C .

2-Psychrophilic bacteria :-the optimum temperature for this bacteria 10-15°C and these temperature 5-30 °C.

3-Thermophilic bacteria :-bacteria present in the habitat which is temperature between 55-60°C the temperature rang 40-80°C.

**4-Classification according to nutritional requirement divided into :-**

1-Simple nutrition:-which need simple requirement of growth factor for growth.

2-Complex nutrition:-which need different type of organic source example vitamin , enzyme , hormone.

**5-Morphological classification :-**

1-Higher bacteria which form filaments and branch or mycelia these group include actenomycosis and some $G^+$ bacteria .

2-True bacteria which form filaments or mycelia when group this bacteria include $G^+$ and $G^-$ bacteria (bacilli and cocci and vebrio (Vibiro , spirosheds). (spirochetes)

# Sterilization, disinfectant and antiseptic

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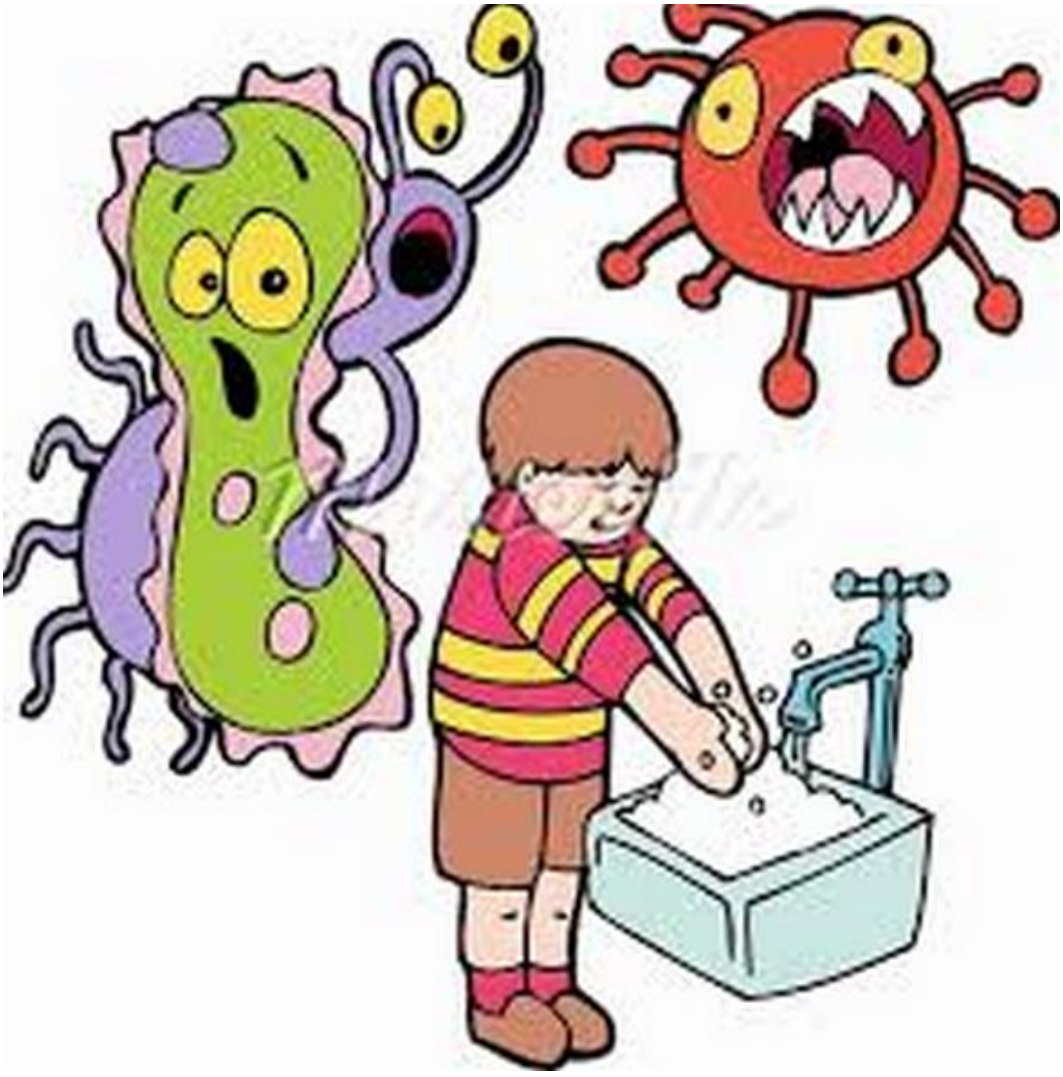


**Sterilization:-**It is **killing of all living forms** of microbes including spores.

**Disinfectants**:- are antimicrobial agents that are applied to non-living objects to destroy microorganisms . (Reducing the number of pathogenic microorganisms)**.**

**Antiseptics:-** are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection , sepsis , or putrefaction.

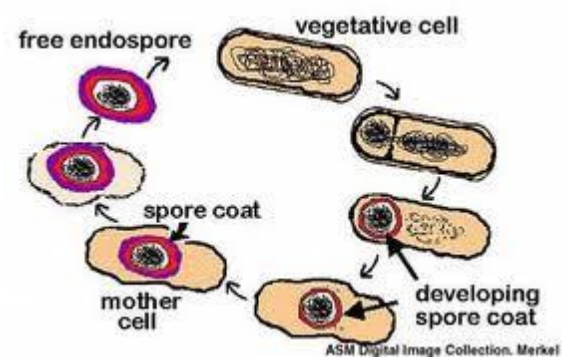
| **Note** That not all disinfectants are antiseptics because an antiseptic additionally must not be so harsh that it damages living tissue. |
|---|

**Cleaning:-** is the removal of visible debris, blood and saliva from an instrument, reducing the number of - but not all micro-organisms.

**Bacteriostatic Agent:** An agent that ***inhibits*** the growth of bacteria, but does not necessarily kill them.

**Bactericide**:An agent that kills bacteria. Most do not kill Endospores.

**Sporicide**: An agent that kills spores.




## Sterilization methods

**Sterilization methods are divided into:-**

1-Chemical methods (Cold sterilization) include gas and liquid sterilization.

2-Physical sterilization method include

1-Dry heat (Red heat, hot air oven, incineration)

2-Moist heat (Autoclave)

3-Radiation: (Ionizing and in-ionizing radiation)

4- Infiltration (Fluid and air filteration)

## I-Chemical sterilization method

Chemical sterilization is typically used for devices that would be sensitive to the high heat used in steam sterilization, and for devices that may be damaged by irradiation (rubbers and plastics can become more brittle after irradiation)**.**

**Chemical sterilization method**

Chemical sterilization method :- it is divided into

1- Chemical liquid sterilization

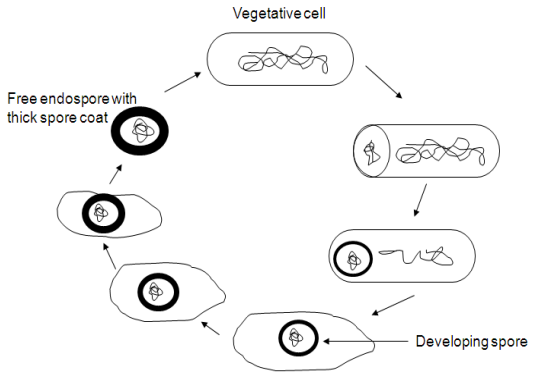
2- Chemical vapour sterilization.

**1- Chemical liquid sterilization**

Disinfectants classification into:

**High level disinfectants**. Use for large number of spores after prolonged exposure , Vegetative bacteria ,Tubercle bacilli ,Fungi ,Viruses example $H_2O_2$(3-6%)

**Intermediate level disinfectants.** Use for Few number of spores ,Vegetative bacteria, Tubercle bacilli ,Fungi ,Enveloped viruses (HBV, HIV). Example 70% ethyl alcohol and isopropyl alcohol.

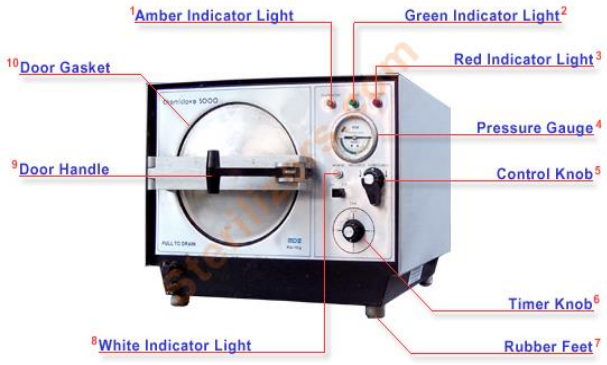



Vegetative and spore form

**Low level disinfectants**. Use for Mainly vegetative bacteria, Some fungi , Narrow range of viruses eample Providon(Iodine 5 and 10%).

**2- Chemical vapour sterilization include**

**1-Chemical Vapor under pressure (Chemiclave):-** This process uses a mixture of chemicals, including alcohol, formaldehyde, ketone, acetone, and water, that are heated under pressure to form a sterilizing gas. Sterilization requires 20 minutes at 131° C and 20 lbs pressure when instruments are either unwrapped or bagged.



Chemiclave

**2- Fumigation** :- to produce the fumigant, potassium permanganate should be mixed with formalin in a ratio (w/v) of 2:3. When the correct ratio of formalin and potassium permanganate is used.

## II- Physical sterilization method

**Physical sterilization method is used for sterilization of:**

1-To sterilise Forceps, Scissors, Scalpels, Swabs.

2-Pharmaceuticals products like Liquid paraffin, dusting powder and fats.

## 1-Heat (Dry and moist sterilization)

## A-Dry heat sterilization

**Mechanisms of action** (1) Protein denaturation, (2) Oxidative damage, (3) Toxic effect of elevated electrolyte (in absence of water).

**Examples of dry heat are:**

**1-Red heat (flaming):**Sterilization of the bacteriological loop by heating in the Bunsen flame or electric incinerator till becomes hot red.


Electric incinerator

**2-Hot air oven:**

1-Temperature of 160$^{o}$C for 2 h. or 170 $^{o}$C for 1 h.

2-For glassware, and metallic instruments.

**3-Incineration:**

This is an excellent method of destroying materials such as contaminated cloth, animal carcasses and pathological materials.

**4- Infrared rays** (common uses: heat glassware and metallic instruments).

## B-Moist heat sterilization

**Principle:**

Kills microorganisms by **coagulating** their proteins.

**Moist heat sterilization** :- Moist heat acts by denaturation and coagulation of protein, breakage of DNA strands, and loss of functional integrity of cell membrane and it is carried out with following methods**:-**

**A-Temperature below 100$^{o}$C.**

**1-Pasteurisation**:-Is process of killing of pathogens in the milk but does not sterilize it (Milk is heated at 63$^{o}$C for 30 mins. Or At 72$^{o}$C for 15-20 sec. Rapid cooling to 13$^{o}$C .

**2-Hot water bath include:-**

A-Vaccine bath: (vaccine sterilization) The contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at 60$^{o}$C for one hour. Only vegetative bacteria are killed and spores survive.

B-Serum bath: The contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at 56$^{o}$C for one hour on several successive days. Proteins in the serum will coagulate at higher temperature. Only vegetative bacteria are killed and spores survive.

**3-Inspissation**: This is a technique to solidify as well as disinfect egg and serum containing media. The medium containing serum or egg are placed in the slopes of an inspissator and heated at 80-85$^{o}$C for 30 minutes on three successive days. On the first day, the vegetative bacteria would die and those spores that germinate by next day are then killed the following day. The process depends on germination of spores in between inspissation. If the spores fail to germinate then this technique cannot be considered sterilization.

**B-Temperature at 100$^{o}$C:**

1-Boiling: Boiling at 100°C for 30 minutes is done in a water bath. Syringes, rubber goods and surgical instruments may be

sterilized by this method. All bacteria and certain spores are killed. (Kills vegetative forms of bacterial pathogens).

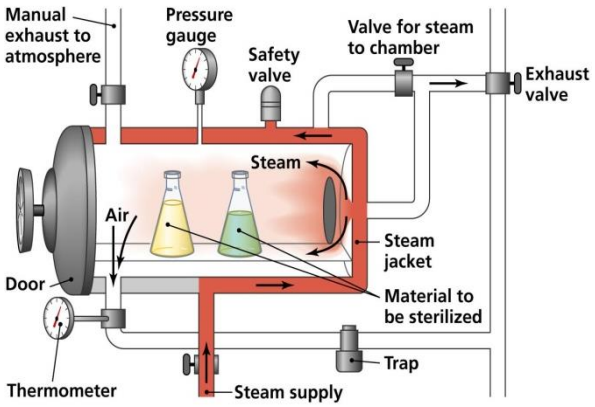
2-Steam :-It works at 100°C under normal atmospheric pressure i.e. without extra pressure. It is ideally suitable for sterilizing media which may be damaged at a temperature higher than 100°C.

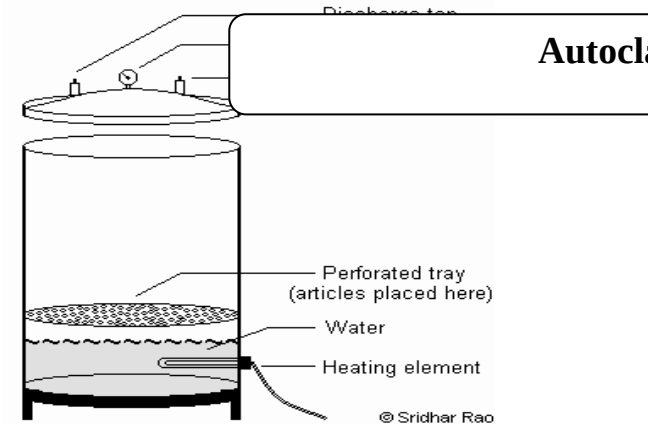
(a)**Single Exposure** for 1.5 hours. It leads to disinfection.

(b) **Tyndallization** Heat labile media like those containing sugar, milk, gelatin can be sterilized by this method. Steaming at 100°C is done in steam sterilizer for 20 minutes followed by incubation at 37°C overnight. This procedure is repeated for another 2 successive days. That is 'steaming' is done for 3 successive days. Spores, if any, germinate to vegetative bacteria during incubation and are destroyed during steaming on second and third day. It leads to sterilization.

C- **Temperature up to 100$^{o}$C (Steam under pressure: Autoclave)**.

Autoclaving is one of the most common methods of sterilization. Principle: When the autoclave is closed and made air-tight, and water starts boiling, the inside pressures increases and now the water boils above 100°C. At 15 Ib pressure, 121°C temperatures is obtained. This is kept for 15 minutes for sterilization to kill spores. It works like a pressure cooker.




**Autoclave is used for sterilization of:**

1-Surgical instruments and dressings.

2-Bed linen.

3-Cotton, gauze

4-Culture media not destroyed by heat.

## **2-Radiation** divided into:-

1- Ionizing 2-Non-ionizing

1- Ionizing rays iclude

- Gamma rays emanate from heated cathode and are employed to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals.

-Gamma rays emanate from nuclear disintegration of certain radioactive isotopes (Co60). kills all bacteria, fungi, viruses and spores. It is used commercially to sterilize disposable petri dishes, plastic syringes, antibiotics, vitamins, hormones, glasswares and fabrics.

**2-Non-ionizing rays**: Rays of wavelength longer than the visible light are non-ionizing. Microbicidal wavelength of UV rays lie in the range of 200-280 nm, with 260 nm being most effective. UV rays induce ultimately inhibits DNA replication. UV readily induces mutations in cells.

**Characteristics**

- Microorganisms such as bacteria, viruses, yeast that are exposed to the effective UV radiation are inactivated within seconds.
- UV rays don't kill spores, they are considered to be of use in surface disinfection.
- UV rays are employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc.
- It doesn't penetrate glass, paper or plastic.

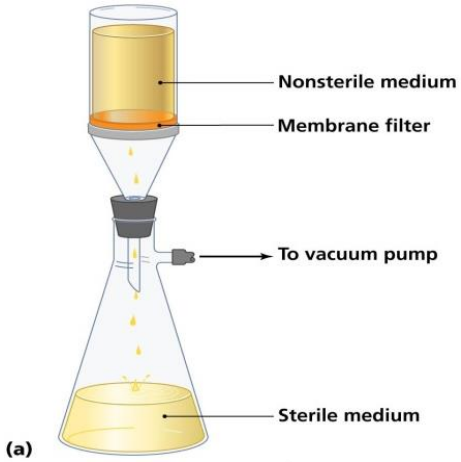
### **3-Sunlight**

The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it. Sunlight is not sporicidal, hence it does not sterilize.

### 4-Filtration

Filtration does not kill microbes, it separates them out. Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydatid fluid.

Filtration is aided by using either positive or negative pressure using vacuum pumps.




## Fluid filters

Used for sterilization of biological fluids destroyed by heat such as serum, plasma, vitamins, hormones.

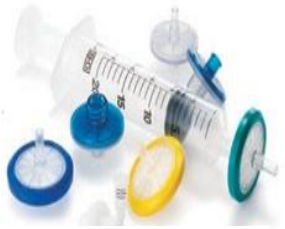
1) **Vacuum or Seitz filter:**

Formed of asbestos disc which is inserted into a metal holder connected to a flask.

2) **Millipore (membrane) filters:**

**Synthetic membranes made from cellulose diacetate.** Membrane filters with pore sizes between 0.2-0.45 µm are commonly used to remove particles from solutions that can't be autoclaved. It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution.

3) **Syringe filter:**



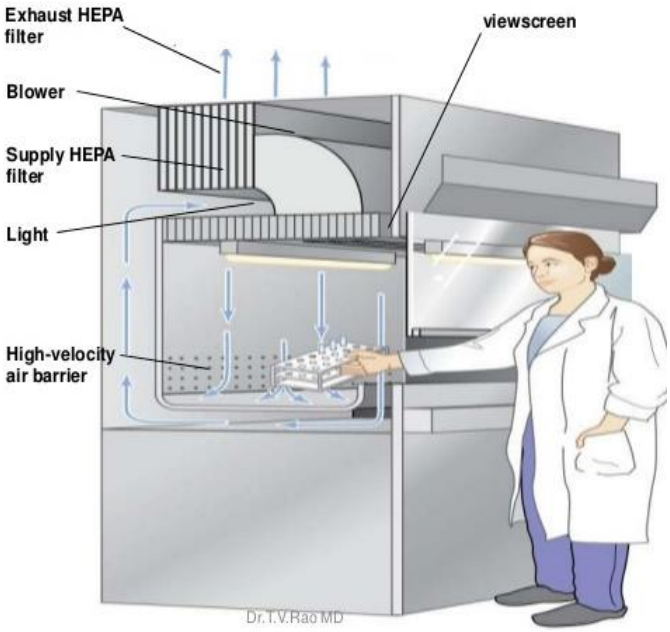
Membrane filters 13-25 µm in diameter in a small holder connected to a syringe containing the fluid to be filtered.

4)- **Air filters**

Air is filtered by HEPA filters.

HEPA means high efficiency particulate air way.

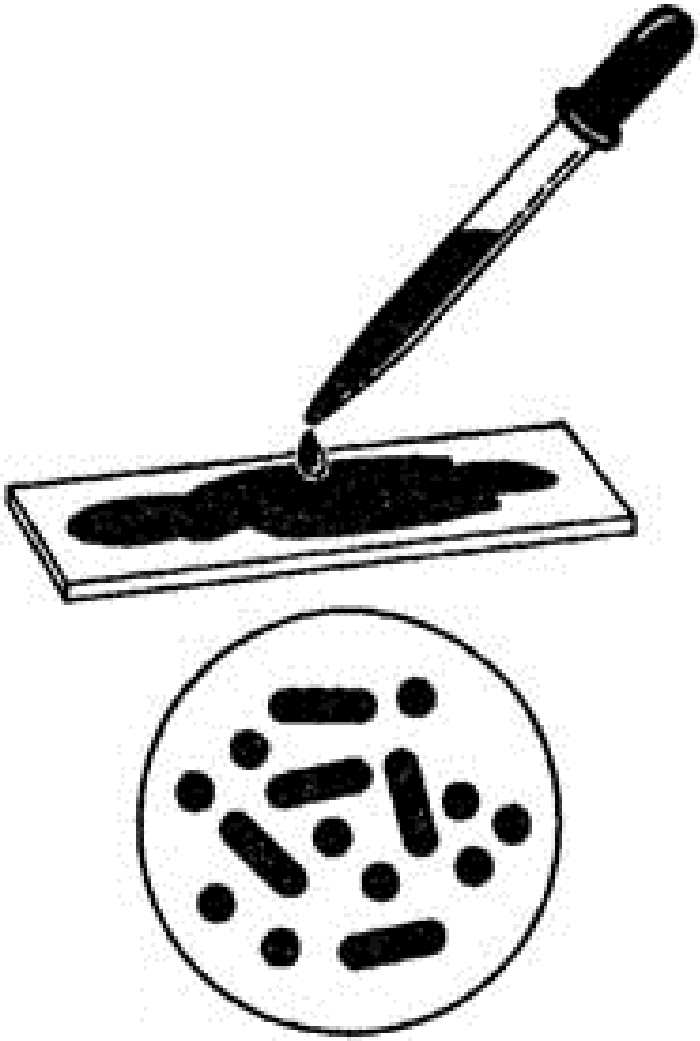
Air filtration is needed in operation rooms, safety cabinets.




Safety cabinets

# Bacterial staining

**5**



**Bacterial staining**: - Stains are dyes or reagents used for differential coloring microorganisms in order to observe their structure more clearly under microscope and divided into:-

**1- Simple stain**

**2-Differential stain**

a-Gram stain

b- Acid –Fast stain (Ziehl-Neelsen stain)

**3-Special stain**

a-Spore stain

b-Capsule stain

**What is the purpose of staining bacterial cells?**

1-Morphological – size, shape, arrangement example Simple stain.

2-Differential – cell wall composition example Gram stain and Acid-fast stain.

3-Structural – cell structures example Endospore stain and Capsule stain.

**Preparing a Bacterial Smear**

Prepared of slide before of staining

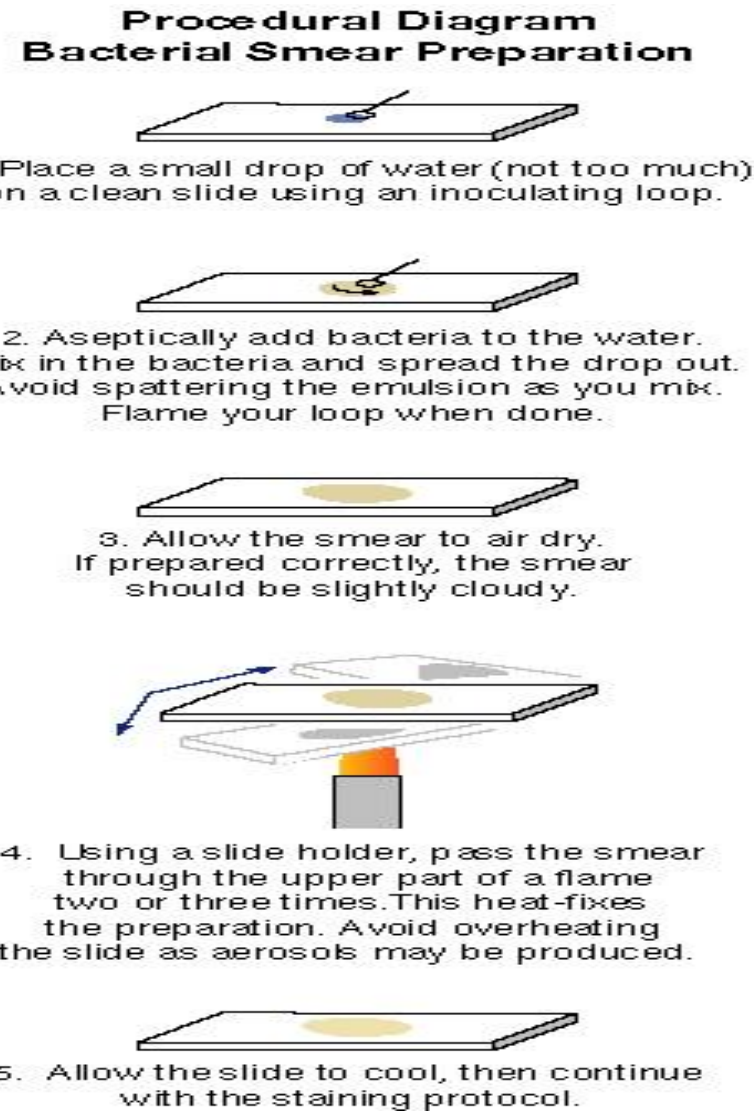
1-Prepare of slide.

2-Added a one drop of sterile saline.

3-Take by loop a small amount of bacterial culture.

4- Mixed the drop of sterile distilled water with culture and spread by the loop on the slide.

5- Dry slide by air or heat (burner).



**1-Simple stain**

**Simple Stain Preparation**

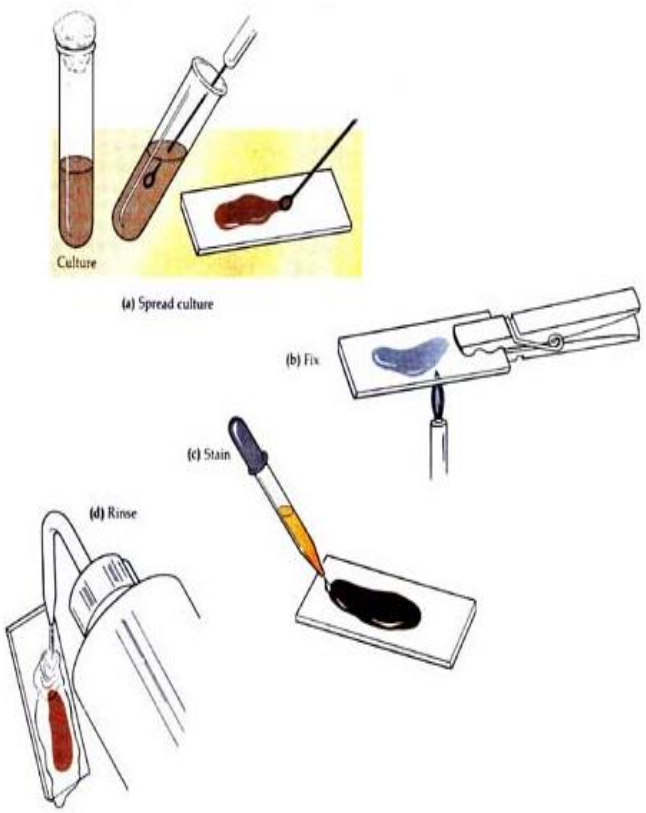
1- Preparing a Bacterial Smear**.**

2- Saturate the smear with basic dye for approximately 1 minute (crystal violet, safranin, or methylene blue).

3- Rinse the slide gently with water.

4- Carefully blot dry with bibulous paper.

5- Observe the slide under the microscope, using proper microscope technique.




2-Differential stain:- it is divided into :-

1- Grams stain 2- Acid fast stain

1- Gram stain is a method of differentiating bacterial species into two large groups (gram-positive and gram-negative).

**Grams stain procedure**

1- Choose an isolated colony off of the agar plate and obtain bacteria with a sterile swab or bacterial loop.

2- Place the swab bacterial loop on the microscope slide and spread the colonies in a circular motion.

3- Heat fix the microorganisms to the slide by placing the bottom of the slide to heat for approximately 30 seconds.

4- Place slide on staining tray or hold with forceps above the sink.

5-Flood the surface of the slide with Crystal Violet stain (Purple stain) and let sit for one minute.

6- Rinse the slide with distilled water.

7- Flood the slide with Gram's Iodine and time for one minute.

8- Rinse the slide with distilled water.

9- Flood the slide with Gram's decolorizer (95% Ethanol alcohol) and time for 30 seconds.

10- Rinse the slide with distilled water.

11- Flood the slide with the counterstain{ Counter-stain Stain applied after decolorization to provide contrast between cells that were decolorized and those that were not} (Safranin) (red colour), and let sit for one minute.

12- Rinse the slide with distilled water.

13-Blot the slide and read with the oil immersion lens of the microscope. Look for Gram-negative and Gram-positive bacteria.

Result

1- Purple color ----------G+

2-Red color (pink) ---------G-

**Mode of action of Gram stain**

Gram-positive bacteria have a thicker peptidoglycan cell wall than gram-negative bacteria.

gram-negative bacteria contain a layer of lipopolysaccharide as part of their cell wall.

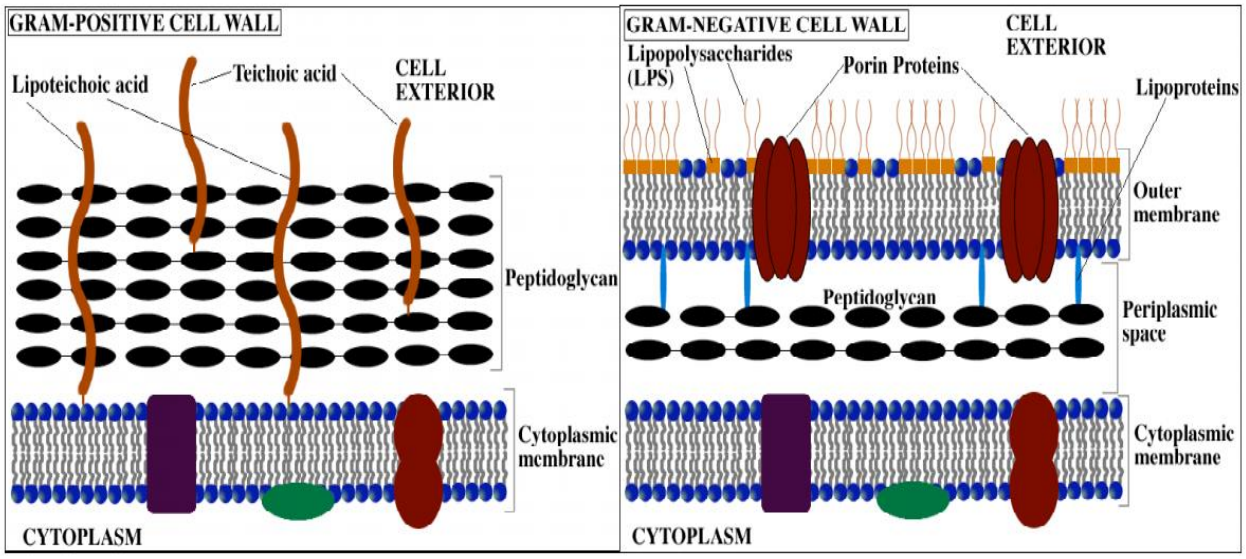
When applied to both gram-positive and gram-negative cells, crystal violet and then iodine readily enter the cells. Inside the cells, the crystal violet and iodine combine to form the crystal violet-iodine (CV- I) complex.

This complex is larger than the crystal violet molecule that entered the cells and because of its size, it cannot be washed out of the intact peptidoglycan layer of gram-positive cells by alcohol. Consequently, gram-positive cells retain the color of the crystal violet dye.

In gram-negative cells, however, the alcohol wash disrupts the outer lipopolysaccharide layer and the CV- I complex is washed out through the thin layer of peptidoglycan . As a result, gram negative cells are colorless until counterstained with safranin, after which they are pink.

Q:- How did you penetrated the gram stain the bacteria cell?

**Cell wall structure of Gram+ and Gram-**




**Gram-positive bacteria**

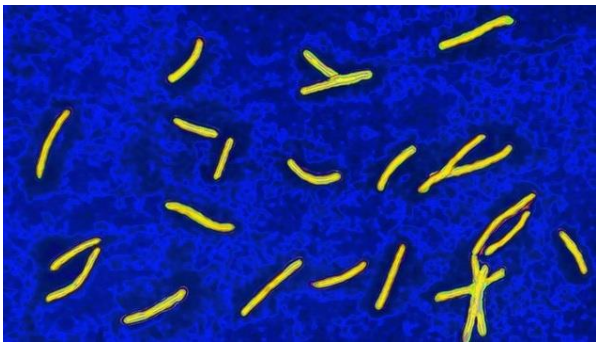
- *Streptococcus*
- *Clostridium*
- *Bacillus*
- *Listeria*
- *Staphylococcus*

**Gram-negative bacteria**

- *Escherichia coli*
- *Helicobacter*
- *Klebsiella*
- *Moraxella*
- *Neisseria*
- *Pasteurella*
- *Proteus*
- *Pseudomonas*
- *Salmonella*
- *Shigella*
- *Vibrio*

**2- Acid –Fast stain (Ziehl-Neelsen stain)**

Use to the *Mycobacterium tuberculosis*.



1. Spread the sputum evenly over the central area of the slide using a continuous rotational movement.
2. Place slides on dryer with smeared surface upwards, and air dry for about 30 minutes.
3. Heat fix dried smear
4. Cover the smear will carbol fuchsin stain
5. Heat the smear until vapour just begins to rise (i.e. about $60^{o}$C). Do not overheat. Allow the heated stain to remain on the slide for 5 minutes.
6. Wash off the stain with clean water.
7. Cover the smear with 3% v/v acid alcohol(3% HCl) + Alcohol) for 5 minutes (or 20% sulfuric acid) or until the smear is sufficiently decolorized, i.e. pale pink
8. Wash well with clean water
9. Cover the stain with Malachite green 5 g/l (0.5% w/v) or Methylene blue, 5g/l
10. stain for 1-2 minutes
11. Wash off stain with clean water
12. Wipe the back of the slide clean, and place it in a draining rack for smear to air dry (do not blot dry).
13. Examine the smear microscopically, using the 100x oil immersion objective and scan the smear systematically.

Note: Heat fixation of untreated specimen will not kill M. tuberculosis whereas alcohol fixation is bactericidal. Acid alcohol is flammable, therefore use it with care. Take great care while heating carbol fuchsin (as staining rack may contain flammable chemicals) to reduce the fire risk.

**Results:**

1. **Acid fast bacilli** : Red, straight or slightly curved rods, occurring singly or in small groups, may appear beaded.
2. Background material: Green

**Mode of action of acid fast stain**

Bacteria with a high wax content(*Mycobacterium tuberculosis*) which do not readily stain with the Gram stain ,retain the primary stain carbol fuchsin when decolorization with acid fast stain

Bacteria with a low wax content(*Pseudomonas aeruginosa*) in their cell wall lose carbolfuchsin and take up the conuterstain methylaine blue these bacteria are called non-acid fast also contain the bacteria mycolic acid in the cell wall of acid fast microorganism is the cytological basis for this differential stain in the phenolic compound . carbolfuchsin is used as

the primary stain because it is lipid soluble and penetrated the wax cell wall.

Special stain is divided into :-

1-Capsule stain 2-Spor stain

1- Capsule stain

**Bacterial capsule** :- is the rigid, slimy and gummy covering of the bacterial cell which lies external to the cell wall.

Uses of Bacterial capsule

1-Protect device against the macrophage and serum (immune).

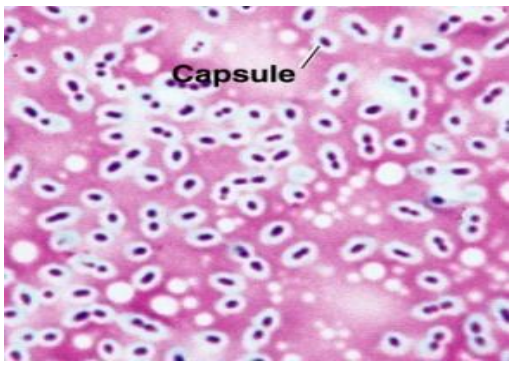
2-Keep the bacteria from external environment.

Capsule staining is divided into :-

1-Hiss stain 2-Anthonys stain

1-His's stain

**Procedure**




1. A smear is prepared on a clean grease free slide.
2. The smear should not be heat fix it should be just air dried.
3. After air drying the smear is flooded with 1 % Crystal violet for about 4 -5 minutes.
4. After 5 minutes the smear is rinse with 20 % copper sulfate solution.
5. Examine the slide under an oil immersion lens. Bacterial cells and the proteinaceous background will appear purplish while the capsules will appear transparent.

2- Anthony's capsule stain

1-prepare the slid without heat fixation

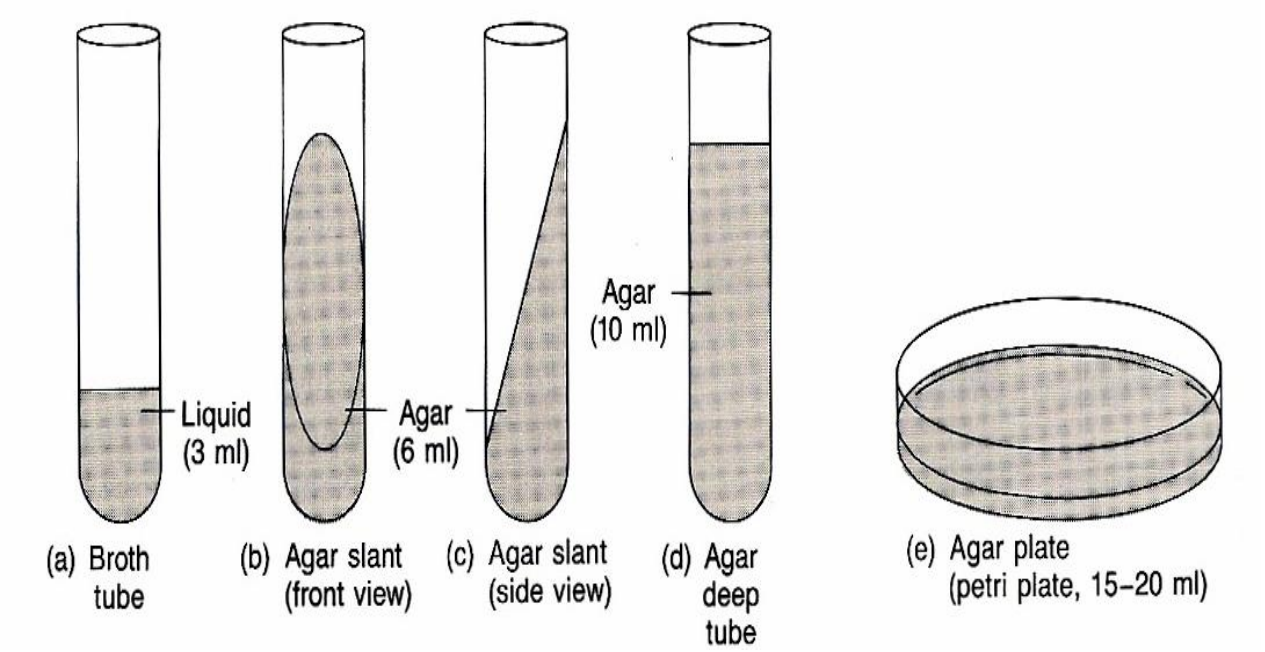
2-Flowed by crystal violate for 3-7 minute
3-Wish with tap water
4-Add 2% copper sulfate
5-Wash for several second
6-Dry by air
7-Examination under microscope (Oil emersion )

2-Spore stain
Spore:- it is a rigid and keratinized membrane surrounded the bacteria in special cases and do not staining by normal stain.
Procedure
1- Prepare the bacterial film with fix by heat
2- Flood the smear with concentrated carbolfuchsin for 5-10 minute(Avoid dry or boiling of stain )
3-Cold the slide
4-Wash with water
5-Dicolorazation by 3%Acetic acid
6-Wash by tap Water for 10-20 second
7-Staining by methyl blue for 1 minute
8-Wash by water
9-Examination under microscope (Oil emersion)

Mode of action
When staining by contrast carbolfuchsin this stain ability penetrated the spore and color with red stain and do not exit this stain when add by acetic acid.

# Bacteria culture media

6



**Culture medium or growth medium** is a liquid or gel designed to support the growth of microorganisms.

**Classification of Bacteria culture medium**

Bacterial culture media can be classified in at least three ways:-

1-Based on consistency
2-Based on nutritional component
3-Based on its functional use

**1-Classification based on consistency:**

Consistency culture media are divided into:-

1- Liquid media
2-Solid media
3-Semi-solid media

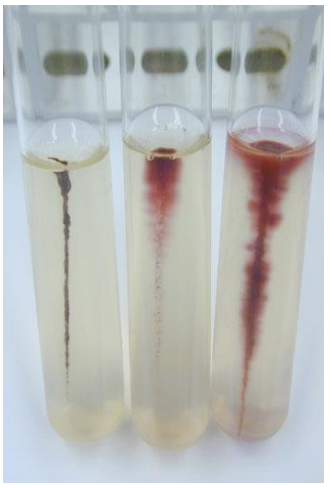
**1-Liquid media (No agar)**: These are available for use in test-tubes, bottles or flasks. Example Nutrient broth. It is used to for inoculum preparation, Blood culture, for the isolation of pathogens from a mixture.

**2-Solid media**:- (Contains 2% agar )any liquid medium can be rendered by the addition of certain solidifying agents (Agar) . Example Nutrient age , Blood agar .

**3-Solid media** is used to colony morphology, pigmentation, hemolysis can be appreciated.

**Note :-** Agar is an unbranched polysaccharide obtained from the cell membranes of some species of red algae and characterized by :-

1. Used for preparing solid medium.
2. Obtained from seaweeds.
3. No nutritive value.
4. Not affected by the growth of the bacteria.
5. Melts at 98$^{o}$C & sets at 42$^{o}$C.
6. 2% agar is employed in solid medium.



3- **Semi-solid media**:- Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid. Such media are fairly soft and are useful in demonstrating bacterial motility example mannitol motility medium .

## 2-Classification based on nutritional component

This media can be classified as 1-Simple 2-Complex 3-Synthetic .

**1-Simple media** such as peptone water, nutrient agar and nutrient broth can support most non-fastidious bacteria.

**2-Complex media** such as blood agar have ingredients whose exact components are difficult to estimate.

**3-Synthetic or defined media** such as Davis & Mingioli medium are specially prepared media for research purposes where the composition of every component is well known.

## 3-Classification based on functional use or application

Include :-

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

2- **Enriched media**: Addition of extra nutrients in the form of blood, serum, egg yolk , to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar(it is enriched with heat treated blood) and blood agar .

3- **Selective and enrichment media** are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these. Example **selective media** (Mac Conkey's medium which selective for gram negative bacteria and mannitol salt agar selective for gram positive bacteria), while **enrichment media** example (Alkaline Peptone Water – for *Vibrio cholera).*

4- **Differential media or indicator media**: These media contain an indicator which changes its colour when a bacterium grows in them. Example MacConkey's agar which is differential for lactose fermentation , mannitol and mannitol salt agar which is differential for mannitol fermentation.

5- **Transport media**: Media used for transporting the samples and delicate organisms may not survive the time taken for transporting the specimen without a transport media. Example Stuart's medium – non nutrient soft agar gel containing a reducing agent and Buffered glycerol saline – enteric bacilli.

6- **Anaerobic media** These media are used to grow anaerobic organisms example Robertson's cooked meat medium, Thioglycolate medium.

**Preparation and preservation of culture media**

1-Check up of pH of the medium before autoclaving.

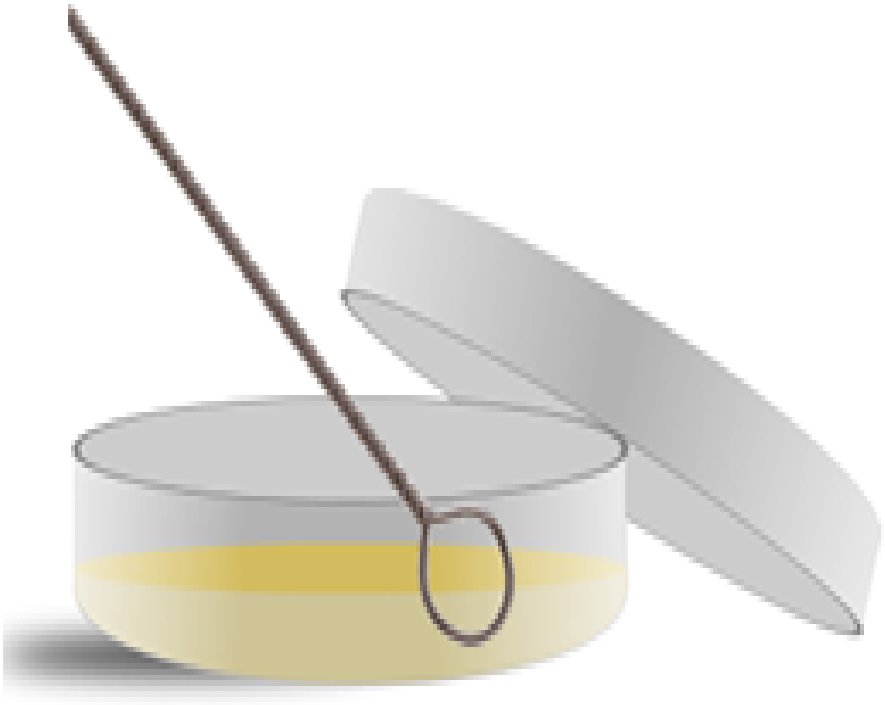
2-Dehydrated media.

3-Most culture media are sterililized by autoclaving. Certain media that contain heat labile components like glucose, antibiotics, urea, serum, blood are not autoclaved. These components are filtered and may be added separately after the medium is autoclaved.

4-Once prepared, media may be held at 4-5$^{o}$C in the refrigerator for 1-2 weeks. Certain liquid media in screw capped bottles or tubes or cotton plugged can be held at room temperature for weeks.

# Ways of culturing bacteria

7

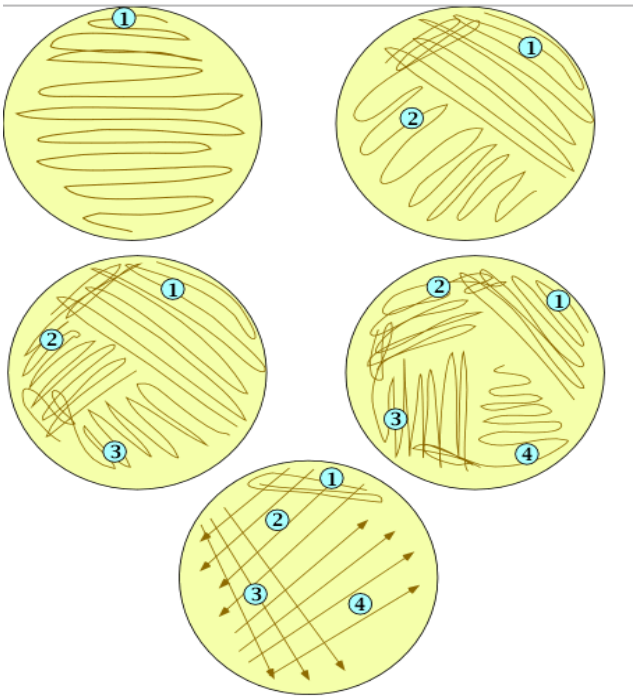


A **microbiological culture** or **microbial culture** is a method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions.

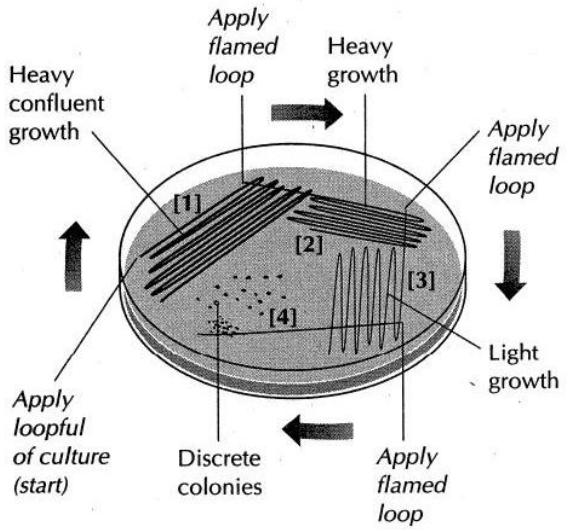
**Bacterial culture methods include**:

1. Streak culture method
2. Lawn culture method
3. Stroke culture method
4. Stab culture method
5. Pour plate method
6. Liquid culture method
7. Anaerobic culture methods

**1-Streak culture method**



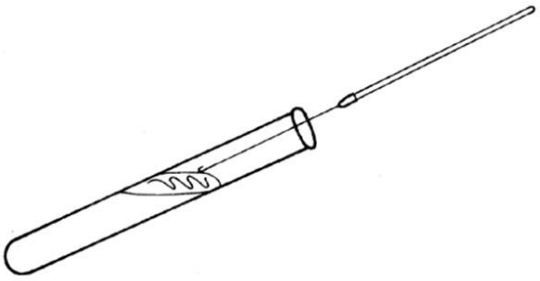
1. Used for the isolation of bacteria in pure culture from clinical specimens.
2. Platinum wire or Nichrome wire is used.
3. One loopful of the specimen is transferred onto the surface of a well dried plate.
4. Spread over a small area at the periphery.
5. The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
6. On incubation, separated colonies are obtained over the last series of streaks.
7. Incubation at 37$^{o}$C for 24 hours(h).



**2-Lawn culture method**

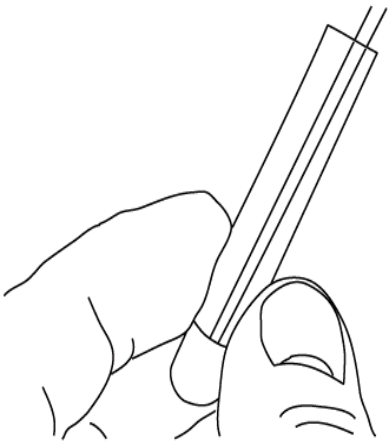
1. Provides a uniform surface growth of the bacterium.
2. Uses
    a. For bacteriophage typing.
    b. Antibiotic sensitivity testing.
    c. In the preparation of bacterial antigens and vaccines.
3. Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium.

**3-Stroke culture method**



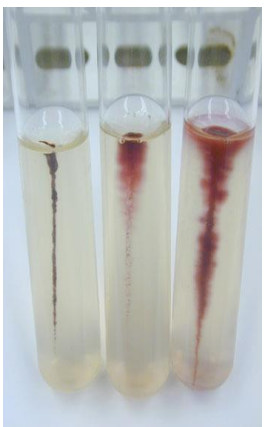
1. Stroke culture is made in tubes containing agar slope / slant.
2. Uses
    a. Provide a pure growth of bacterium for slide agglutination and other diagnostic tests.

**4-Liquid cultures method**



1. Liquid cultures are inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.
2. Uses
    a. Blood culture
    b. Sterility tests
    c. Continuous culture methods
3. Disadvantage
    a. It does not provide a pure culture from mixed inocula.

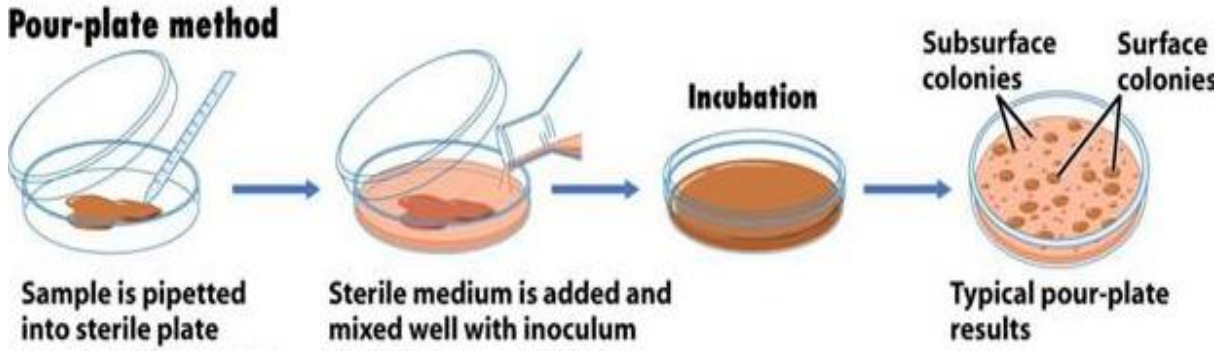
**5-Stab culture method**



1. Prepared by puncturing a suitable medium – gelatin or glucose agar with a long, straight, charged wire.
2. Uses
    a. Demonstration of gelatin liquefaction.
    b. Oxygen requirements of the bacterium under study.
    c. Maintenance of stoke cultures.

**6-Pour plate culture**

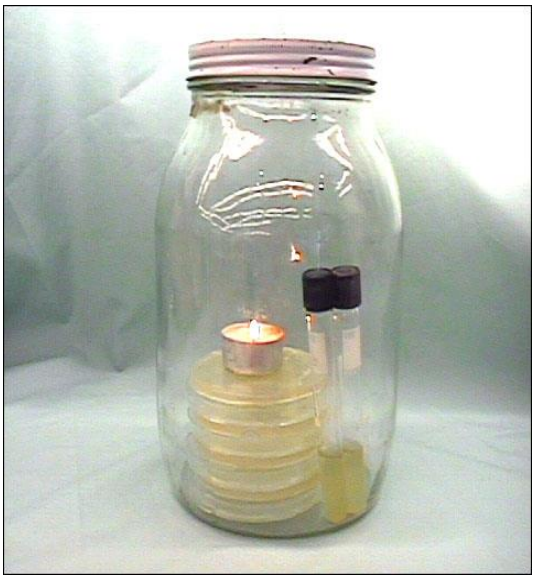
1. Agar medium is melted (15 ml) and cooled to 45$^{o}$C.
2. 1 ml of the inoculum is added to the molten agar.
3. Mix well and pour to a sterile petri dish.
4. Allow it to set.
5. Incubate at 37$^{o}$C, colonies will be distributed throughout the depth of the medium.
6. Uses
    a. Gives an estimate of the viable bacterial count in a suspension.

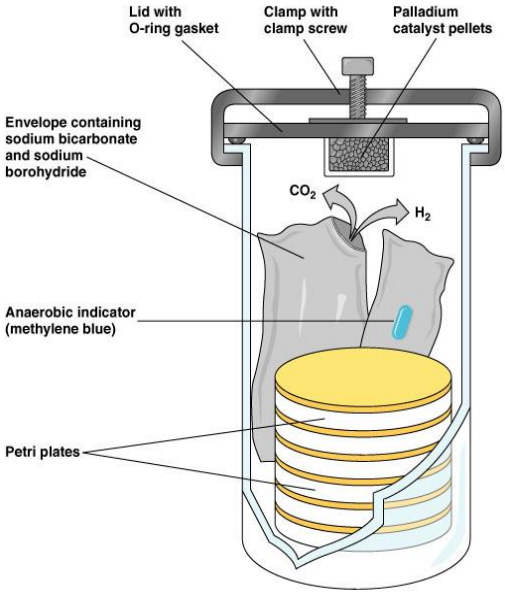



    b. For the quantitative urine cultures.

**7-Anaerobic culture methods**

1. Anaerobic bacteria differ in their requirement and sensitivity to oxygen.
2. *Closteredia tetani* is a strict anaerobe – grows at an oxygen tension < 2 mm Hg.
3. **Methods:**
    a. Production of vacuum
    b. Displacement of oxygen with other gases
    c. Chemical method
    d. Biological method
    e. Reduction of medium
    f. **Production of vacuum:**
4. Incubate the cultures in a vacuum desiccator.
    a. **Displacement of oxygen with other gases**
5. Displacement of oxygen with hydrogen, nitrogen, helium or $CO_2$.
6. Candle jar




Lid with
O-ring gasket
Clamp with
clamp screw
Palladium
catalyst pellets
Envelope containing
sodium bicarbonate
and sodium
borohydride
$CO_2$
$H_2$
Anaerobic indicator
(methylene blue)
Petri plates

# Heamatology

8

**Hematology**

**1**-Blood (Functions, properties, composition).
**2**-Blood plasma
**3**-Blood serum
**4**-Erythrocyte (properties,shapes,number,functions) production and degradation of blood cells.
**5**- Leukocyte (types, shapes, number, functions).
**6**- Platelets (number, functions, Coagulation of blood, anticoagulant).
**7**- Hemoglobin (functions, normal value, composition).
**8**- Blood groups (types, Rh factor).

**Heamatology: -** it is a branch of medical science dealing with the blood and blood-forming tissues, including morphology, physiology, and pathology.

# Blood

**Blood**:-The circulating fluid (plasma) and suspended formed elements, such as Red Blood Cells (RBCs), White Blood Cells (WBCs) and platelets in the vascular system of humans and other vertebrates.

**Functions of Blood**

1. Transport of nutrition.
2. Transport of respiratory gases.
3. Acts as a vehicle.
4. Drainage of Waste Products.
5. Blood clotting.
6. Regulation of body temperature.
7. Defense actions.
8. Regulates blood pressure.
9. **Maintains pH balance inside the body.**
10. Regulation of Body Fluid Electrolytes.

**Composition of blood**

Blood consist of:-

**1-C**ellular material (99% red blood cells, with white blood cells and platelets making up the remainder) and 55% plasma.

**2-**Water **3-**Amino acids **4-**Proteins **5-**Carbohydrates

**6-**Lipids **7-**Hormones **8-**Vitamins **9-**Electrolytes

**10**-Dissolved gases **11**-Cellular wastes

**Blood formation**

**Haemopoiesis**:-It is the production of the formed elements of blood.

**Haemopoietic tissues** refer to the tissues that produce blood.

**Production of Blood**

**In the fetus**

**1**-The earliest haemopoietic tissue to develop is the yolk sac, which also functions in the transfer of yolk nutrients of the embryo.

**2**-In the foetus, blood cells are produced by the bone marrow, liver, spleen, and thymus.

**3-In the adult**

RBCs (Red blood cells) are manufactured in the red bone marrow of bones. In adults, RBCs are only manufactured in specific bones; thoracic, vertebrae, cranial, and the ends of femurs and humerous bones.

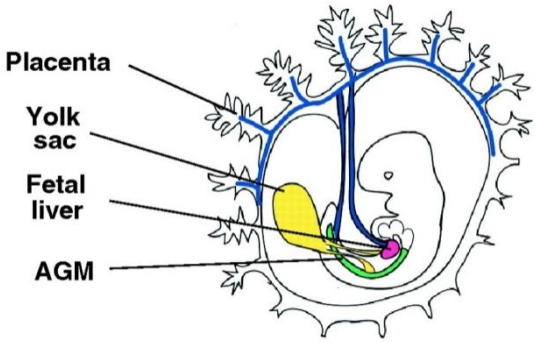



**Figure (5):-** Yolk sac of embryo **(this fee for information only)**.

**Plasma: -** Plasma is the straw-colored liquid in which the blood cells are suspended.

**Structure of plasma**

Normal blood plasma is 90-92 % water.
This is the straw-colored fluid in which the blood cells are suspended, and consists of:-

1-Dissolved substances including electrolytes such as sodium, chlorine, potassiun, manganese, and calcium ions.

2- Blood plasma proteins (albumin, globulin, and fibrinogen).

3- Hormones

**Plasma functions**

**1**- The medium in which the blood cells are transported around the body (by the blood vessels) and are able to operate effectively.

**2**- Maintain optimum body temperature throughout the organism.

**3**- Helps to control the pH of the blood and the body tissues, maintaining this within a range (7.3-7.4) .

**4**- Helps to maintain an ideal balance of electrolytes in the blood and tissues of the body.

**Serum:-**The clear, pale-yellow liquid that can be separated from clotted blood.

**Serum includes**

1-All proteins not used in blood clotting (coagulation).
2-All the electrolytes.
3-Antibodies
4-Antigens
5-Hormones

## Erythrocytes (Red blood cells)

**Erythrocytes (Red blood cells).** A cell that contains hemoglobin and can carry oxygen to the body. Also called a red blood cell (RBC).

**Erythropoesis**

Erythropoiesis refers specifically to the production of erythrocytes or red blood cells (RBCs).

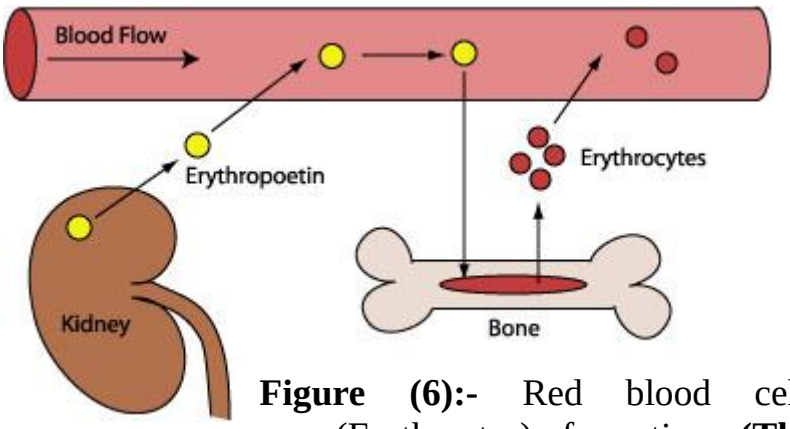



**Figure (6):-** Red blood cells (Erythroctes) formation **(This fee is required).**

**Functions of RBCs**

1. The red blood cells transport oxygen from the lungs to the tissues in the body.
2. They also carry carbon dioxide from the body tissues to the lungs.
3. Adhere to the walls of blood vessels at the site of an injury and thus plug the defect in the vascular wall.
4. Blood clotting.

**Shape of RBCs**

RBCs are round discs which are bi-concave that lack a cell nucleus. This allows them to be flexible, and twist and turn through all the blood vessels easily.

**Normal value**

Male: 4.7 - 6.1 x $10^6$ cells /$cmm^3$.
Female: 4.2 - 5.4 x$10^6$ cells /$cmm^3$.

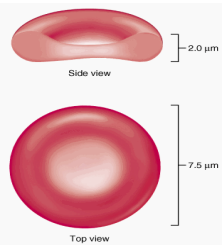


**Figure (7):-**Erythrocyte shape **(this fee for information only)**.

**Degradation of RBCs**

RBCs are dyeing after 120 days and engulfing by macrophage cells. By the lysosomal enzymes macrophage the RBCs degradation into:-

**1**- Heme

2- Globin

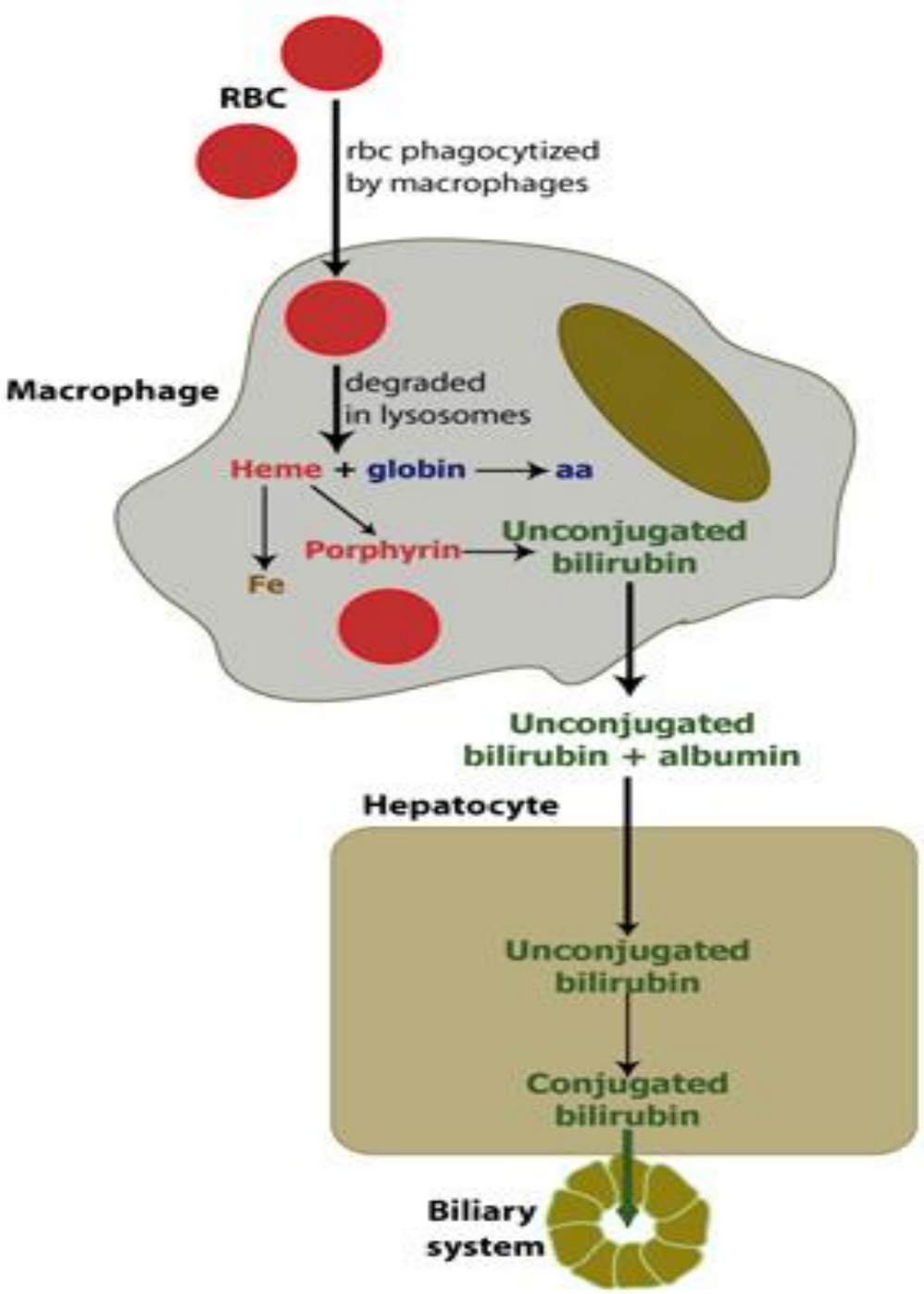


**Figure (7):-**RBCs degradation **(This fee is required).**

**White blood cells (or leucocytes or leukocytes):-** it is blood cells that have a nucleus and cytoplasm and help protect the body from infection and diseases.

**White blood cells properties**

1-It is have nuclei and do not contain hemoglobin.
2-Typical concentration is 5,000 - 9,000/cmm$^3$.
3- White blood cells (also called leukocytes) are fewer in number than red blood cells.
4- They are capable of Amoeboid Movement.

**Functions of WBCs**

1- Kill and eat bacteria and fungi.
2-Protect against viral infection.
3- Destroy cancer cells.
4- Remove dead or damaged cells.
5- Allergic response.
6- Develop antibodies.

**Types of WBCs:- there are two types of WBCs**

**I-Granular white blood cells**:- these leukocytes characterized by the presence of differently staining granules in their cytoplasm when viewed under light microscopy and include:

1-Neutrophils

a-It is representing about 50 - 70% of WBCs.
b- It is Phagocytose, or ingests, bacteria and fungi.
c- It is a segmented nucleus that forms two to five lobes.
d- Their cytoplasm is packed with pale granules.
e- It is the first of the WBCs to arrive at the site of an injury (1$^{st}$ line defense).
f- It is lifetime about 6 hours–few days.

2-Eosinophils

a- It is representing about 1 - 4% of WBCs.
b- Their granules stain darkly with eosin, a red dye.
c- It is a segmented nucleus that forms two lobes.
d- It is lifetime about 8–12 days.
e- Their granules which appear a fine, faintly pink color.

f- It is stimulant in the parasitic infection and in the allergic inflammatory responses.

3-Basophils

a-It is representing about less than 1% of WBCs.

b- It is secrete anti-coagulant and vasodilator substances as histamines.

c- It is a segmented nucleus that forms two or three lobes.

d- Their granules which appear a large size and blue color.

e- It is lifetime about a few hours to a few days.

**II-Agranular (or non-granular) white blood cells**:- It is leukocytes characterized by the apparent absence of granules in their cytoplasm.

1-Lymphocytes

a- It is representing about 25 - 40% of WBCs.

b- There are divided into two types:-

a- Small lymphocyte.

b-Large lymphocyte.

c-It is a specific immune (T and B lymphocyte).

d- See just a thin halo of cytoplasm around a relatively large, round nucleus.

2-Monocytes

a- It is representing about 2 - 8% of WBCs.

b- It is a nucleus is appearing as kidney shaped.

c- It is lifetime about hours to days.

d-Monocytes quickly migrate from the blood vessel and start an intense phagocytory activity.

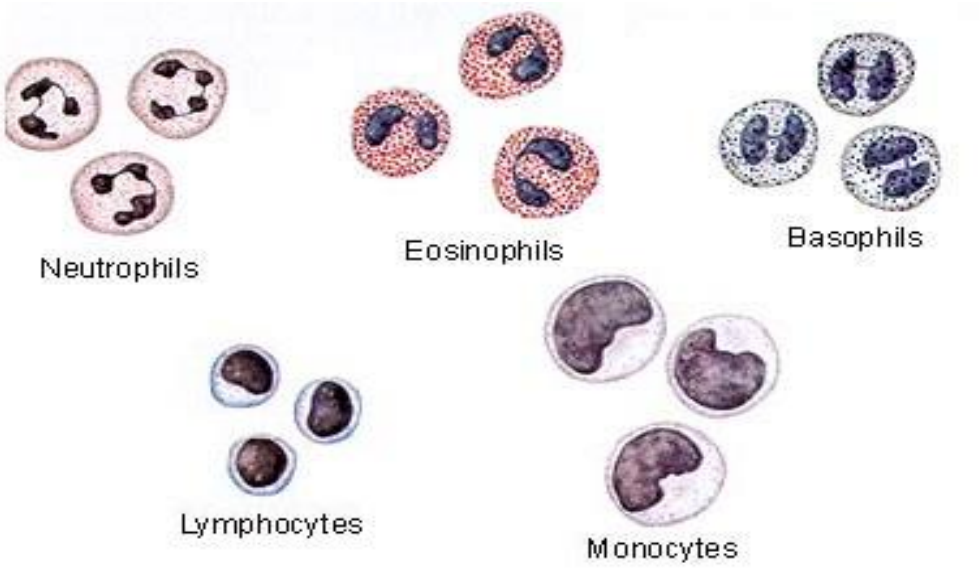
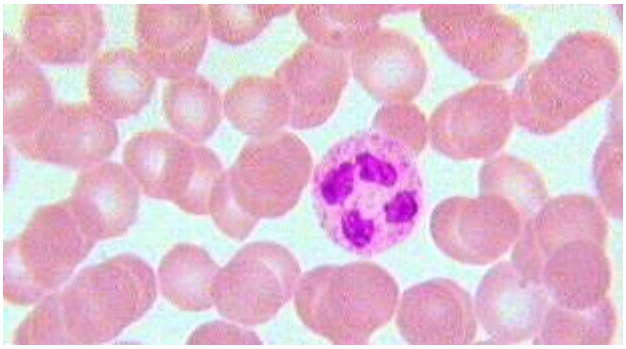
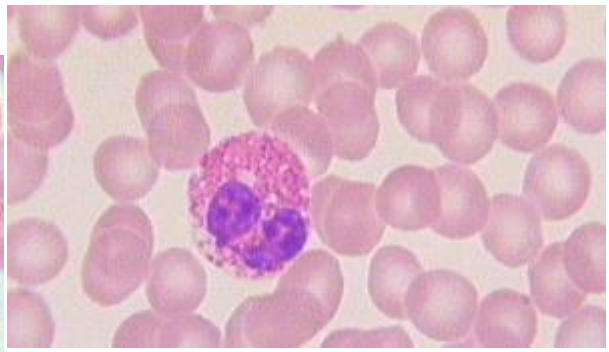
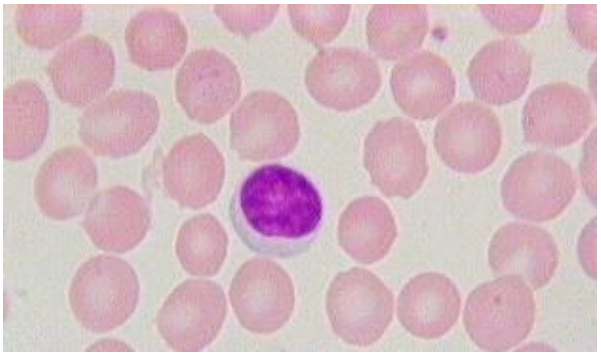


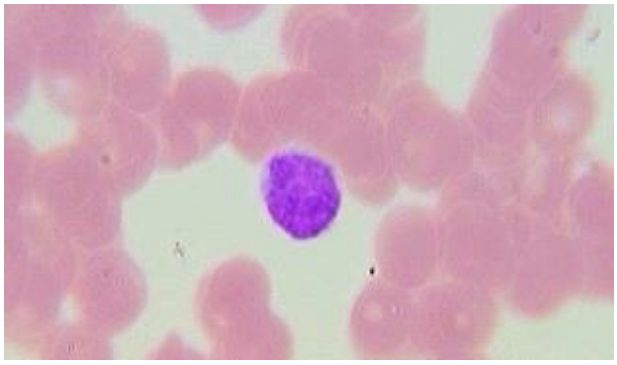
Figure (8):- Different types of WBCs **(This fee is required).**

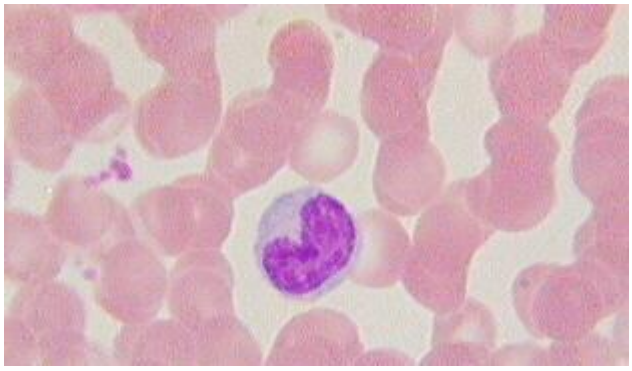
Leucopenia:- It is decrease number of white blood cells.
Leukocytosis:-It is increase number of white blood cells.











# Thrombocytes (Platelets)

**Platelets, or thrombocytes** are small, irregularly shaped clear cell fragments (cells that do not have a nucleus), 2-3 µm in diameter, which are derived from fragmentation of precursor megakaryocytes.

**Shape of blood platelets**

Disk-shaped fragments.

**Thrombocytes characteristics**

1-Diameter 2-4 um (Have many granules but no nucleus).

2-Have longevity of approximately 5-9 days.

3-There are approx. 150,000 - 400,000 platelets per micro-liter of blood.

4- Platelets originate from cells known as megakaryocytes.

**Functions of throbocytes**

To facilitate blood clotting - the purpose of which is to prevent loss of body fluids.

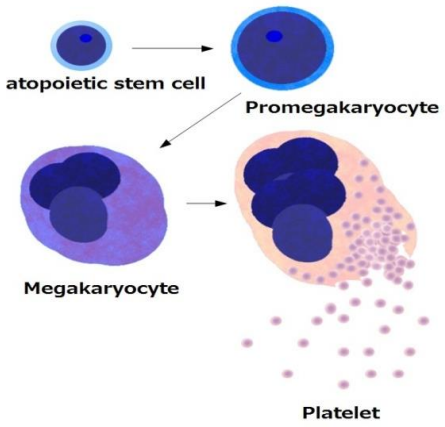



**Figure (9)**:-Platelet formation **(This fee is required).**

## Clotting factaors

Any of the chemical compounds in the blood which interact to create the coagulation process, or blood clotting. Factors are designated by Roman numerals and names include.

Factor I (fibrinogen)
Factor II (prothrombin)
Factor III (tissue thromboplastin)
Factor IV (calcium)
Factor V (proaccelerin)
Factor VI (no longer considered active in hemostasis)
Factor VII (proconvertin)
Factor VIII (antihemophilic factor)
Factor IX (plasma thromboplastin component; Christmas factor)
Factor X (stuart factor)
Factor XI (plasma thromboplastin antecedent)
Factor XII (hageman factor)
Factor XIII (fibrin stabilizing factor).

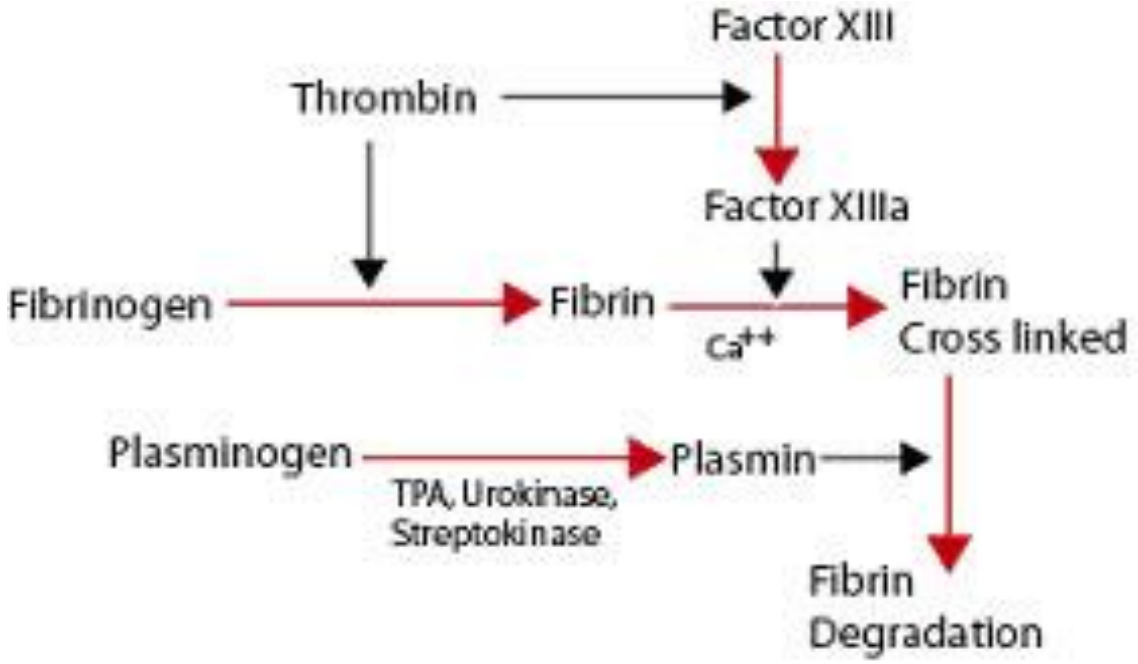
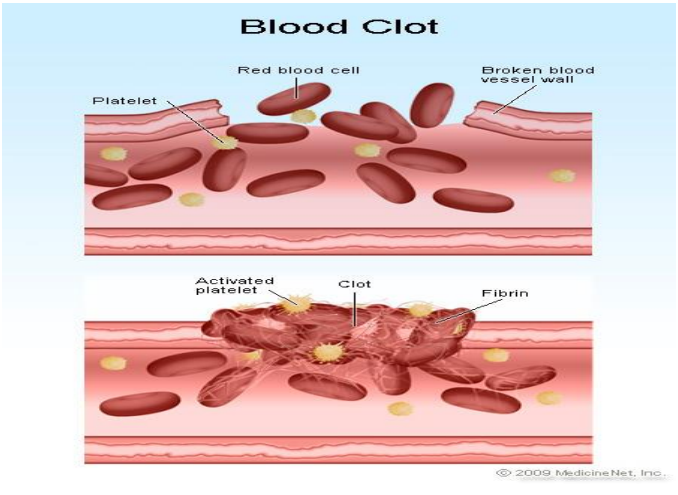
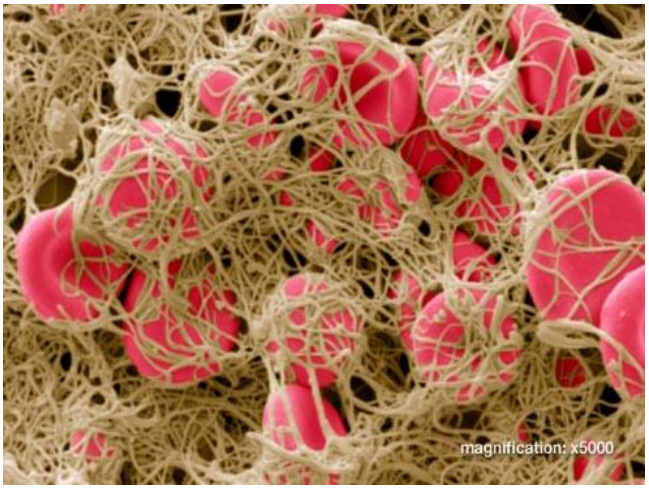



**Figure (10):-** Mechanism of thrombosis formation **(This fee is**



**required)**.

**Figure (11):-** Clotting formation **(This fee for information only).**

**Blood groups**

The membranes of human RBCs are containing two specific Antigens (Ag).It is divided into:-

1-ABO system 2-Rh system

**The ABO system**

There are 4 major types of blood groups:-

**Table (1):-**ABO system.

| Blood group | Antigen |
|---|---|
| Group **A** | Is contain **A**- antigen only |
| Group **B** | Is contain **B**- antigen only |
| Group **AB** | Is contain **A** and **B**- antigen |
| Group **O** | When neither **A** nor **B** is present |

**Rh system**

The Rh factor named for the **Rhesus** monkey. The **Rh** factor is considering a specific antigen present only in the RBCs is called **D-antigen.**

**Rh factor is divided the human into:-**

1-Rh (-ve) is present in human at a ratio 15%.

2-Rh (+ve) is present in human at a ratio 85%.

**Uses of blood groups**

To transfusion of blood from person to other person without lead to coagulation of blood in human body.

**Table (2):** ABO groups.

| **Blood group** | **Antigens on RBCs** | **Antibodies in serum** |
|---|---|---|
| Group **A** | Is contain **A**- antigen only | Serum is contain **Anti-b** |
| Group **B** | Is contain **B**- antigen only | Serum is contain **Anti-a** |
| Group **AB** | Is contain **A** and **B**- antigen | None |

| | | |
|---|---|---|
| Group **O** | When neither **A** nor **B** is present | Serum is contain **Anti-a** and **Anti-b** |

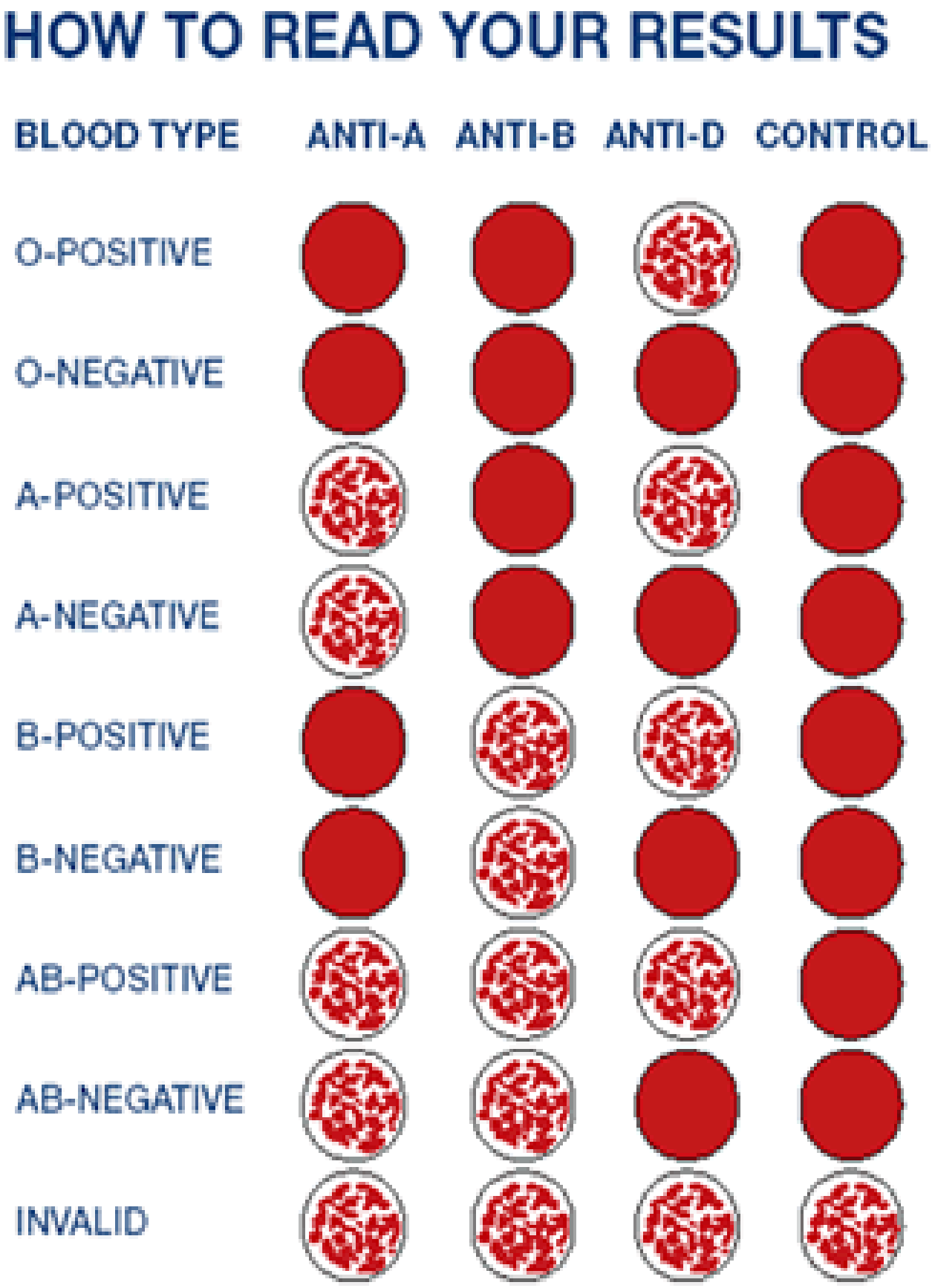
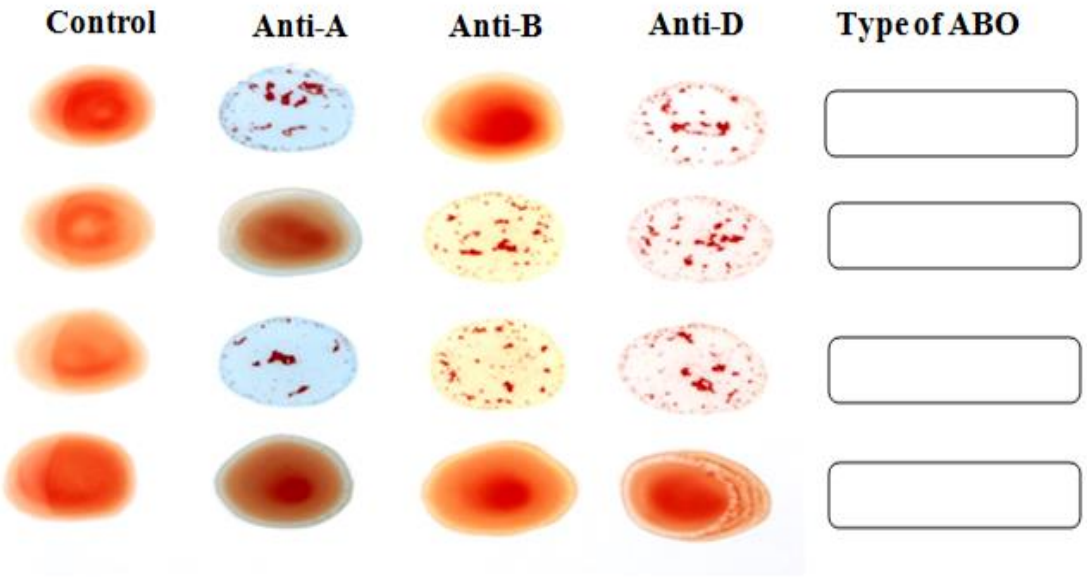



**Figure (11):- Different ABO groups and Rh.**




**Hemoglobin** is a protein in red blood cells (RBCs) that carries oxygen from the lungs to the tissues in the body. The pigment in hemoglobin is responsible for the red color of blood.

**Functions of Hemoglobin**

1-It carries oxygen from the lungs to the body tissues and takes carbon dioxide from the tissues to the lungs.
2- It gives the red color to the blood

**Components of hemoglobin**

The name **hemoglobin** is made from :
1-Globin is the globular protein
2-Heme-an iron atom
The common type of **hemoglobin** consists of four subunits:
Two alpha (α) subunits or globins
Two beta (β) subunits or globins

**Types of hemoglobin**

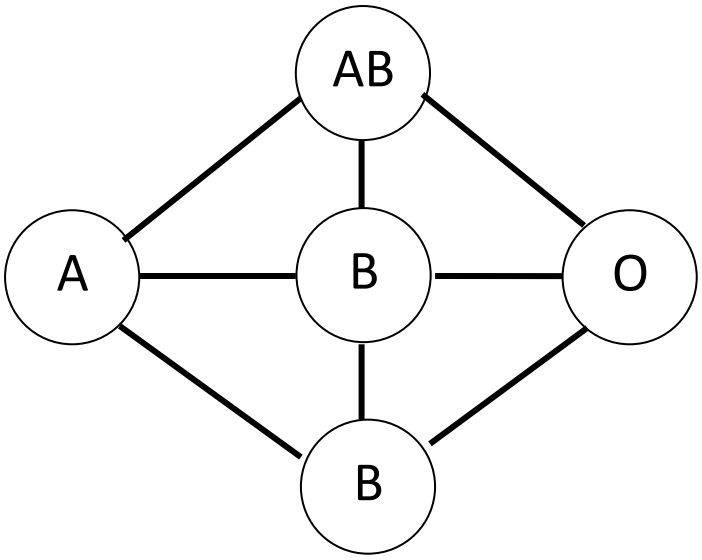
1-Hb A composes about 95 to 98% of Hb found in body and contains two alpha protein chains and two beta protein chains.

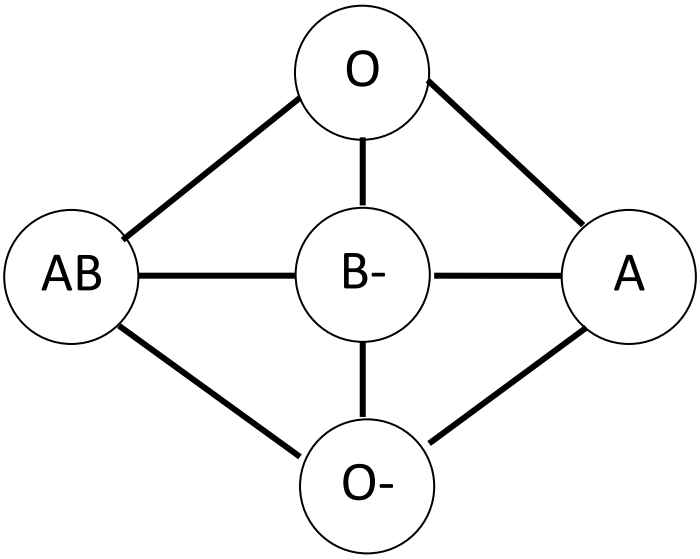
2-Hb A2 makes up a smaller portion -- about 2% of the Hb in body. It has two alpha and two delta protein chains.

3-Hb F makes up 2% of the Hb found in your body. It has two alpha and two gamma protein chains.

**Normal value of Hb**

In adults: - **12 -15 g/dl**


AB
A
B
O
B


O
AB
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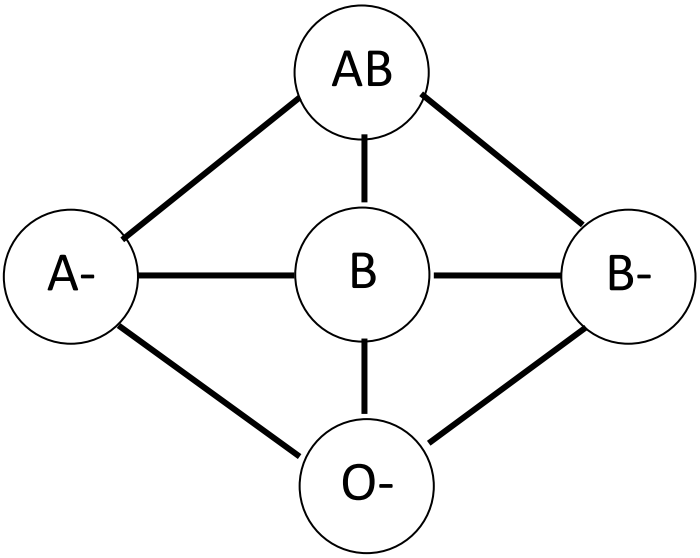
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# Anticoagulants and Haematological tests

9

# Anticoagulants

**Anticoagulants** (antithrombics) are a class of drugs that work to prevent the coagulation (clotting) of blood.

**Anticoagulants uses**

1-Anticoagulants reduce blood clotting which can help prevent deep vein thrombosis, pulmonary embolism, myocardial infarction and ischemic stroke.

2- Some anticoagulants are used in medical equipment, such as test tubes, blood transfusion bags, and renal dialysis equipment.

**Anticoagulants** agents

1- Heparin 2-Warfarin 3-Sodium citrate

4- Ethylenediamine tetraacetic acid EDTA

# Hematological tests

Common Hematology Laboratory Tests

1-Complete Blood Count (CBC):- is one of the most commonly ordered blood tests.

And includes:

RBC count
WBC count
Platelet count

2-Hemoglobin

13-Hematocrit

4- Differential WBCs

5-Erythrocyte Sedimentation Rate (ESR)

6-Coagulation Tests include:

-Bleeding time
-Clotting time

**1-Complete Blood Count (CBC) includes:**

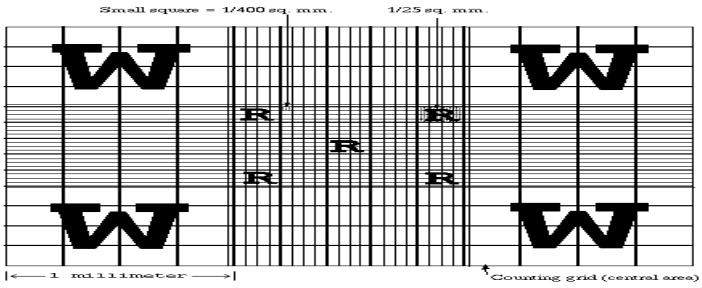
-RBC count
-WBC count
-Platelet count


**Hemocytometer Chamber**

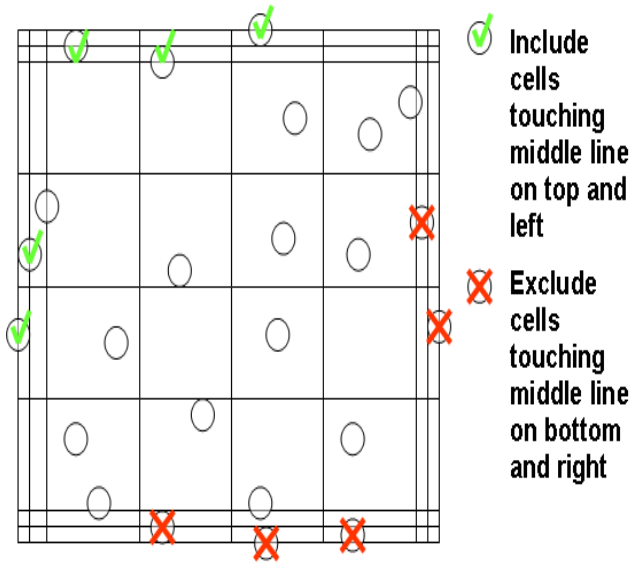
**Blood Cells counts requirements**

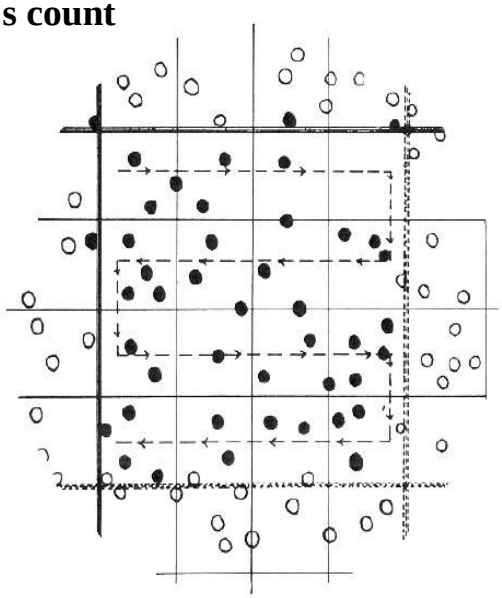
1- Hemocytometer Chamber

2- Pipette
3- Special solutions
4- Microscope
5- Cover slide

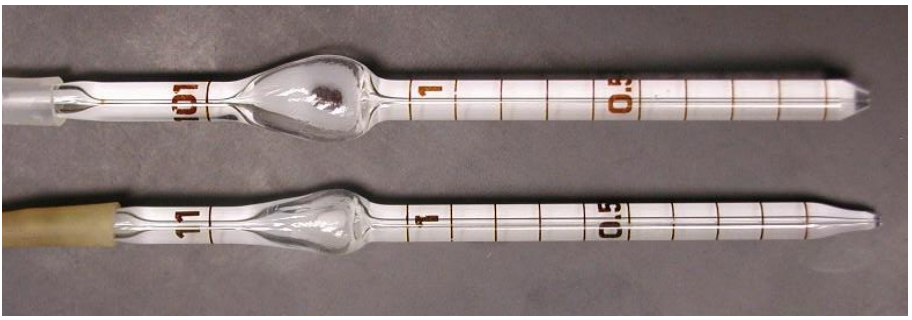



**W=WBCs count site**
**R=RBCs count**



**Mechanical way of Blood cells count**



**RBCs count requirements**

1- Hemocytometer Chamber
2- Pipette (contain on red bead)
3- Special solutions (Hayem's solution).
4- Microscope
5- Cover slide

**RBCs count=**N X $10^4$ Cell/$mm^3$

**N** = the total number of red cells counted in the counting chamber.

**WBCs count requirements**

1- Haemocytometer slide
2- Pipette (contain on White bead)
3- Special solutions (Thomas's solution).
4- Microscope
5- Cover slide

**WBCs count=** N X 50 Cell/$mm^3$

**N** = the total number of white cells counted in the counting chamber.

**Platelet count**

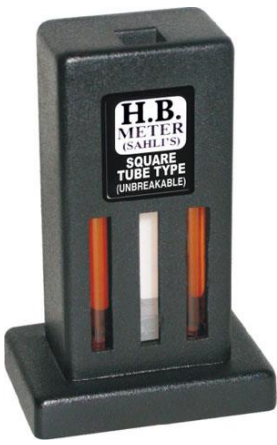
1- Haemocytometer slide
2- Pipette (contain on red bead)
3- Special solutions (ammonium oxalate solution 1%).
4- Microscope
5- Cover slide

Platelet count = N X $10^3$ Cell/$mm^3$

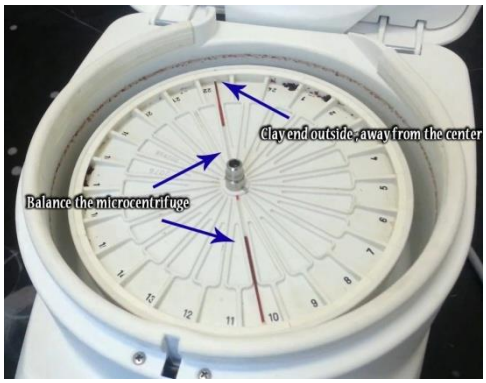
N= Total number of platelets cells (Count 4 white blood cells squares).

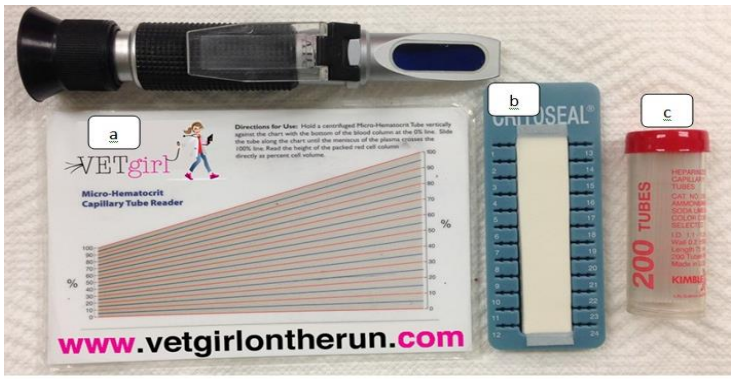
**Hemoglobin test**

1- Sahli's haemoglobinometer.
2. Two Pasteur pipettes (one for HCl and one for distilled water).
3. Glass rod to stir ( stirrer )
4. 0.1 N - Hydrochloric acid
5. Distilled Water
6. Comparison tube.
7. Pipette( Hemoglobin pipette with rubber tubing and mouthpiece)




**Hematocrit test (Packet cell volume {PCV}):-**is the volume percentage (%) of red blood cells in blood.




Microcentrifuge a=PCV reader , b=Clay , c=Capillary(Heparinized)

**Purposes**

Blood is made up of red and white blood cells, and plasma. A decrease i nthe number or size of red cells also decreases the amount of space they occupy, resulting in a lower hematocrit. An increase in the number or si ze ofred cells increases the amount of space they occupy, resulting in a h igher hematocrit.

**Polycythemia**, cause an overproduction of redblood cells, resulting in a n increased hematocrit.

**Anemia**, cause a decrease of red blood cells, resulting in an ~~derease~~ hematocrit.

Normal range for women: 36 - 46%

Normal range for men: 41 - 53%

**Erythrocyte Sedimentation Rate (ESR):**-is the rate at which red blood cells sediment in a period of one hour.

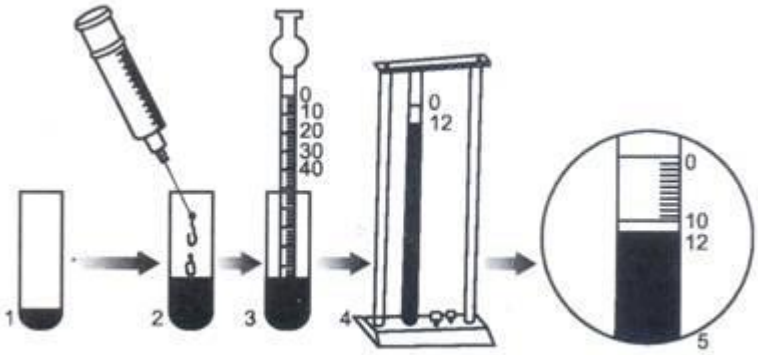



Fig. 9.1: Steps showing Westergren method

The Wintrobe sedimentation rack

Whole blood (4 volumes) diluted with 0.109 M trisodium citrate (1 volume). Alternatively, 3 anticoagulated with EDTA. (If this specimen type blood [2.0ml] must be diluted with 0.85% w/v sodium chloride [0.5ml] prior testing).

**purpose**

It is a common hematology test, and is a non-specific measure of inflammation.

**Normal values:**

Adult male 0-15 mm/hr , Adult female 0-20 mm/hr

**An elevated ESR may be found in**:

1. Pregnancy (after the third month).
2. Acute and chronic infections.
3. Rheumatic fever.
4. Rheumatoid arthritis.
5. Myocardial infection.
6. Nephrosis.
7. Acute hepatitis.
8. Menstruation.
9. Tuberculosis.
10. Hypothyroidism.

11-Hyperthyroidism

**A decreased ESR will be present in**:

1. Polycythemia.
2. Congestive heart failure.
3. Hypofibrinogenemia.

## Coagulation Tests

**Coagulation** is a complex process by which blood forms clots.

**Bleeding time: -** It is measures the time taken for blood vessel constriction and platelet plug formation to occur.


Fig. 18.2: Wright method of clotting time

**Aim**

Measures quality of platelets

Normal value of bleeding time is 1-6 min.

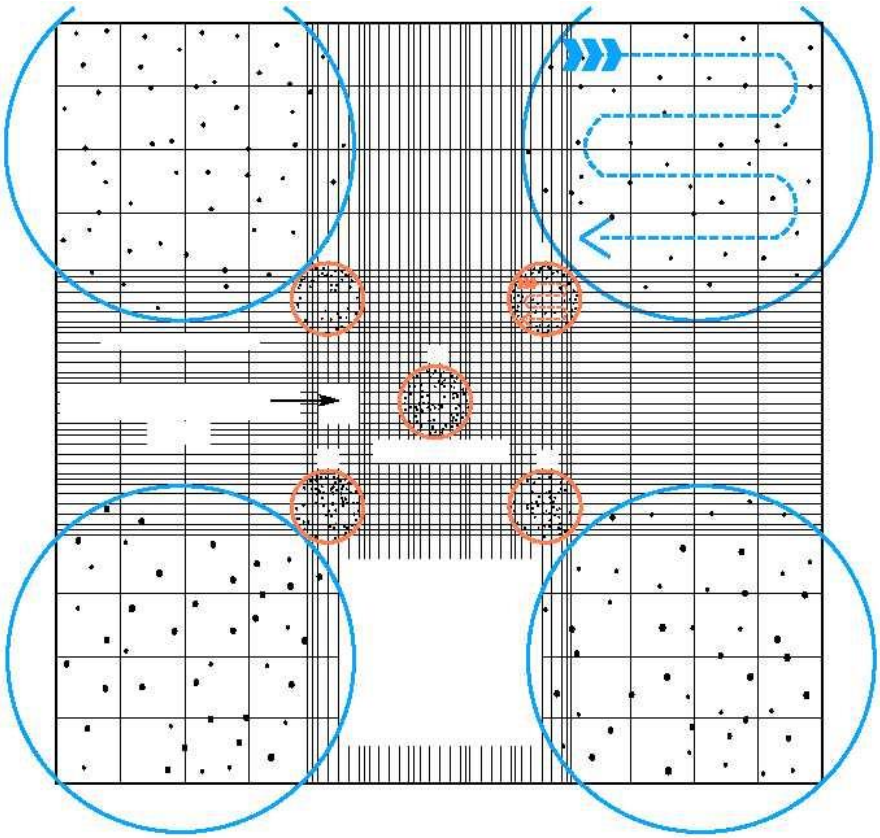
**Clotting time:-** The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner.

**Aim**

Measures quality of prothrombin

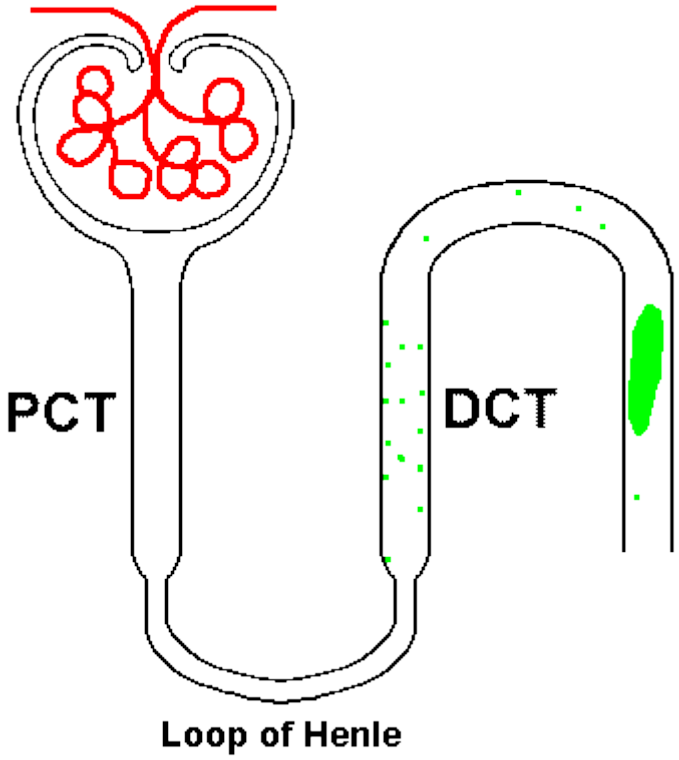
Normal value of clotting time is 2 to 6 minutes.

Exam ple



# Urine Examination

# 10



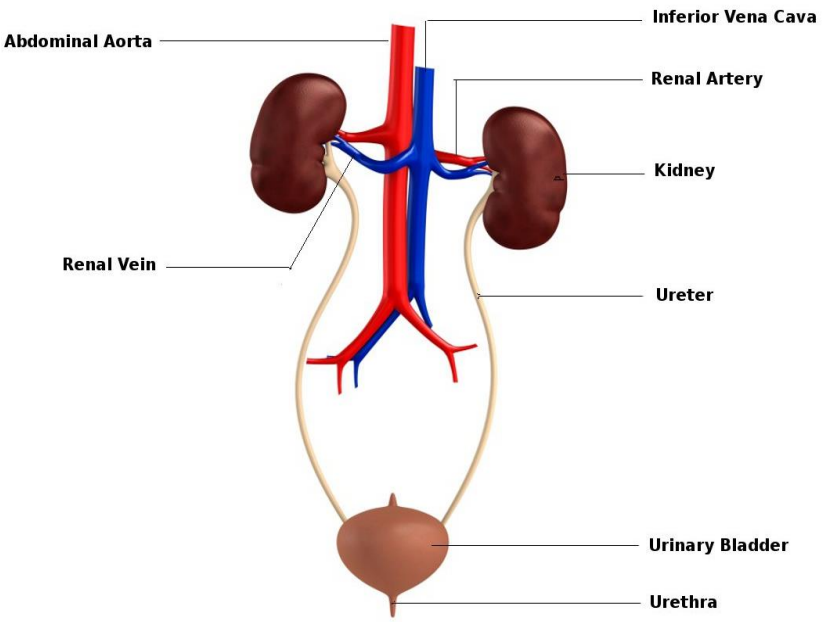
**Urinary system: -** The system that includes all organs involved in reproduction and in the formation and voidance of urine**.**

**Functions of Urinary system**

1-Storage of Urine
2-Excretion of urine
3-Regulation of blood volume.
4-Regulation of erythrocyte production
**5- Regulation of blood pressure**
**6- Regulation of the pH of the blood**
**7-Regulation of the ionic composition of blood**

**Structure of Urinary system**

1-Kidney (Renal)
2-ureter
3-urinary bladder
4-urethra




Human urinary system.

## 1-Kidney (Renal)

The kidneys are a pair of organs that are found on either side of the spine, just below the rib cage in the back.

**Functions of kidney**

1-Execretory function. The kidneys remove waste products from the blood and produce urine.

2-Endocrine function. By secreted the Erythropoietin hormone which is responsible on formation of RBCs.

3-Regulation functions.

a-Water-electrolytes balance.

b-Acid-Base balance.

4- Regulate blood pressure and the levels of water, salts, and minerals in the body.

5- Producing vitamin D, which keeps the bones strong and healthy.

**2- Ureter**

One of the two tubes that carry urine from the kidneys to the bladder.

**3- Urinary bladder**

An elastic, muscular sac situated in the anterior part of the pelvic cavity in which urine collects before excretion.

**4-Urethra**

The urethra is a thin tube that carries urine from the bladder to outside the body.

**Urine: -** It is a fluid that is yellow to amber in color, slightly acidic, and discharged from the body through the urethra.

**Normal urine composition:-**

1-Bilirubin (result from breakdown of haemglobin.
2-Creatinine (from muscle creatine)
3-Urea (result from metabolic of amino acid).
4-Uric acid (result from metabolic of nucleic acid).
5- Ketone bodies
5-water 95%
6-Ions ($K^+$ ,$Na^+$,$Cl^-$)

## Structure of kidney

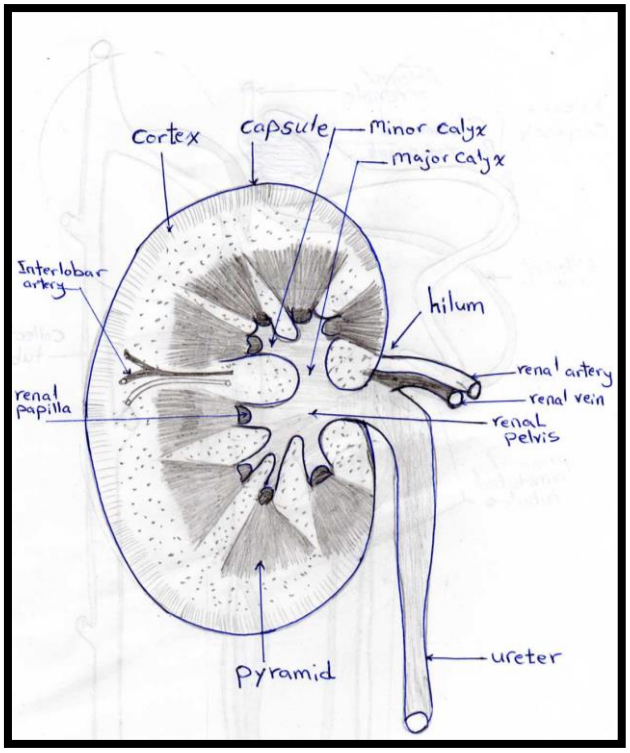
The kidney is composed from two layers:-

**1-Cortex: -** it is characterized by**:-**

1-Outer part of the kidney
2-It is pale in colour.

**2-Medulla:-** it is characterized by:-

1-Inner part of the kidney
2- It is red in colour .
3-The medulla contains 10-15 pyramids.




Cross section of kidney **(This fee is required)**.

**Nephron:-**It is the function unit of the kidney .There is more than 1000.000 nephron in each kidney.

**Types of nephron**

**1-Cortical nephrons:-** these nephrons are located mainly in the cortex and characterized by presence of short loop of henle.

**2-Juxtamedullary nephrons**

These nephrons are located mainly in the medulla of the kidney and characterized by presence of long loop of henle.

## Structure of nephron

**1-Bowman capsule**

Is the initial dilated part which contains an invaginated tuft of capillary vessels of the glomerulus.

**2- Proximal convoluted tubules (PCT).**

**3-Loop of henle.**

Is consisting of:-

1-Dscending limb (thin segment).

2-Ascending limb (thick segment).

**4-Distal convoluted tubules (DCT).**

This tubules affected by 2 hormone.

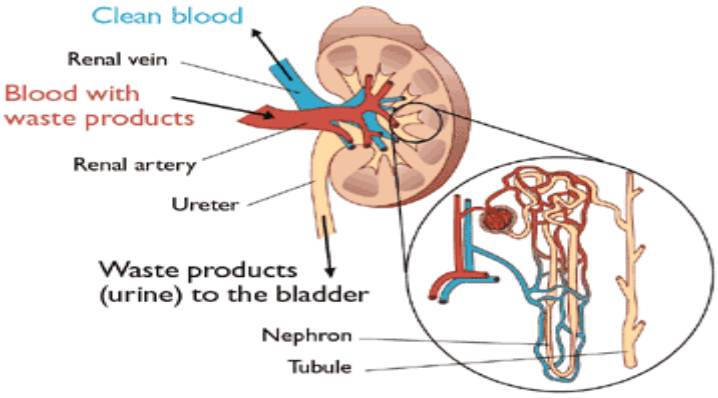
**1-Aldostrone hormone** leads to increase $Na^+$ and water reabsorption from distal convoluted tubules.

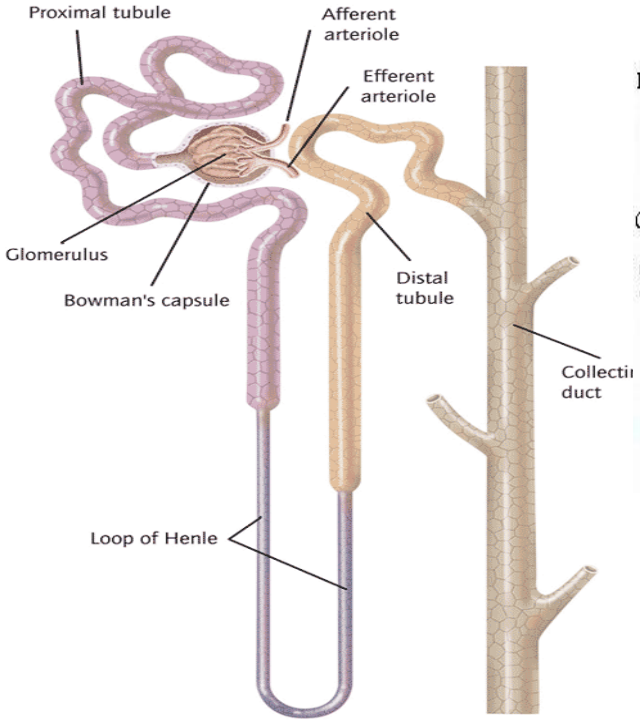
**2-Antidiretic hormone** leads to increase $Na^+$ and water reabsorption from distal convoluted tubules.

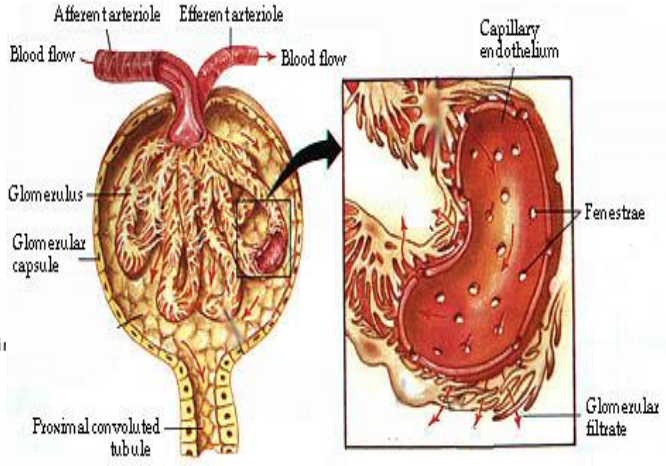
**5-Collecting tubules**.

All 8 distal tubules calculated to form collecting tubule.

Structure of nephron **(this fee for information only)**




Structure of nephron **(this fee for information only)**.

Structure of nephron**(This fee is required)**.

**Functions of nephron**

**1-Filtration**

In the membrane of blood capillaries of the glomerulus, this blood capillaries is contain a pores (diameter 100μm).This pores filtrated the blood from glomerulus to Bowman's lumen.

This filtrated fluid is containing:-

1-**Ions** ($Na^+$,$K^+$,$Ca^{++}$,$Mg^+$,$H^+$,$HCo_3^-$,$Cl^-$ ).

2-**Glucose**

3-**Water**

4-**Metabolic waste (**creatinine ,urea , $NH_4^+$).

**Material that was not filtrated through the glomerulus.**

All blood cells.

**NOTES:-**

All substances or materials which contain molecular weight more than 60.000 Dalton are not filtration from glomerulus to Bowman lumen and include:-

1-Red blood cells 2-White blood cells 3-Protein hormones 4-Plasma proteins.

**2-Re-absorption**

Some of materials reabsorption from lumen tubular nephrons into body and these materials and ions include :-( $Na^+$, $Ca^{++}$, $Cl^-$, $H^+$, $K^+$ and Glucose).

**3-Secretion**

There are certain substances which are secreted by tubular cells into glomerulus filtrated to maintain normal level in the body tissue example ($Na^+$, $Cl^-$, $K^+$, $H^+$, $HCo_3$, and $NH_4$).

**Oligouria:-**It is decrease in urine volume.

**Causes of oligouria.**

**1-**Nephritis.

**2-**Hypotension.

**3-**Heart failure.

**4-**Obstruction calculi or renal stone.

**5-**Renal failure.

**Polyuria:-**It is increase in urine volume.

**Causes of polyuria**

**1-**Drinking high amount of water.

**2-**Some disease example Diabetes mellitus and uremia.

**3-**Lock of antidiuretic hormone leads to increase urine output. This disease is called diabetes insipidus.

# General Urine Exam

Routine Urinalysis (Routine-UA):
It consists of a group of tests performed as part of physical examination. It involves macroscopic and microscopic analysis.

**Time of analysis:**

- must analyzed within 1h at room temp. or within 8hr at $2^{o}C$- $8^{o}C$
- If not assayed within these time limits, several changes will occur.
  - Sample should collected in a **clean container**.
  - Urine container must be **sterile** if the urine is to be cultured.
  - For **microscopic examination**, the urine must be fresh.

**Type of analysis:**

1. Macroscopic analysis: include 1- Physical characteristics 2- chemical analysis
2. Microscopic examination: urine sediment is examined under microscope to identify the components of the urinary sediments.

## Macroscopic analysis

A-Physical examination involves:

1. Color
2. Transparency
3. Odour
4. Volume
5. pH
6. Specific gravity

### Abnormal Physical properties of urine

**A:- Colour**

1. Amber yellow ( **Urochrome)** result derivative of urobilin, produce from bilirubin degradation, is pigment found in normal urine).
2. Colorless due to reduced concentration.
3. Silver or milky appearance result from Pus, bacteria or epithelial cells
4. Reddish brown result from Blood (Hemoglobin).
5. Yellow foam result Bile or medications.
6. Orange, green, blue or red result medications.
7. Vitamin B supplements can turn urine bright yellow.

**2- Transparency:**

- Urine is normally clear. Bacteria, blood, sperm, crystals, or mucus can make urine look cloudy.
- Is classified as clear or turbid.
- **In normal urine:** the main cause of cloudiness is crystals and epithelial cells.
- **In pathological urine:** it is due to pus, blood and bacteria.
- **Degree of cloudiness depends on:** pH and dissolved solids
  - Turbidity: may be due to gross bacteriuria,
  - Smoky appearance: is seen in hematouria.
  - Thread-like cloudiness: is seen in sample full of mucus.

**3- Odour:**

Odour has little diagnostic significance.

1. Aromatic odour------> Normal urine due to aromatic acids.
2. Ammonia odour------> On standing due to decomposition of urea.
3. Fruity odour--------> Diabetes due to the presence of ketones.

❖ Urine does not smell very strong, but has a slightly "nutty" odor. Some diseases cause a change in the odor of urine. For example, an infection with *E. coli* bacteria can cause a bad odor, while diabetes or starvation can cause a sweet, fruity odor.

**4- Volume:**

- Is important part of assessment for fluid balance and kidney functions.
- Adults produce from 750ml-2500ml / 24h, with the average of about 1.5L per person.

**5- pH:**

- pH measure acidicity or alkalinity (basic) of urine
- Normal urine pH: 4.5-8.
- Increased acidity in urine: due to diabetes or medications.
- Urine sample must be fresh
  (On standing urine become alkaline as a result of ammonia liberation due to urea decomposition).
- A urine pH of 4 is strongly acidic, 7 is neutral (neither acidic nor alkaline), and 9 is strongly alkaline.

7. **Specific Gravity (SG):**
   Normal value:-1.016-1.022

**Properties**

- Measures the amount of substances dissolved in urine.
- Indicates how well kidneys are able to adjust amount of water in urine.

- Higher SG: more solid material is dissolved in urine
- When you drink a lot of fluid, your kidneys make urine with a high amount of water in it which has a low specific gravity. When you do not drink fluids, your kidneys make urine with a small amount of water in it which has a high specific gravity.

B-Chemical examination of urine

1. Proteins
2. Sugars
3. Ketone bodies
4. Bilirubin
5. Bile salts
6. Urobilinogen
7. Blood

# Microscopic examination

## Urinary casts

Casts are cylindrical structures composed mainly of mucoprotein which is secreted by epithelial cells lining the loops of Henle, the distal tubules and the collecting ducts.

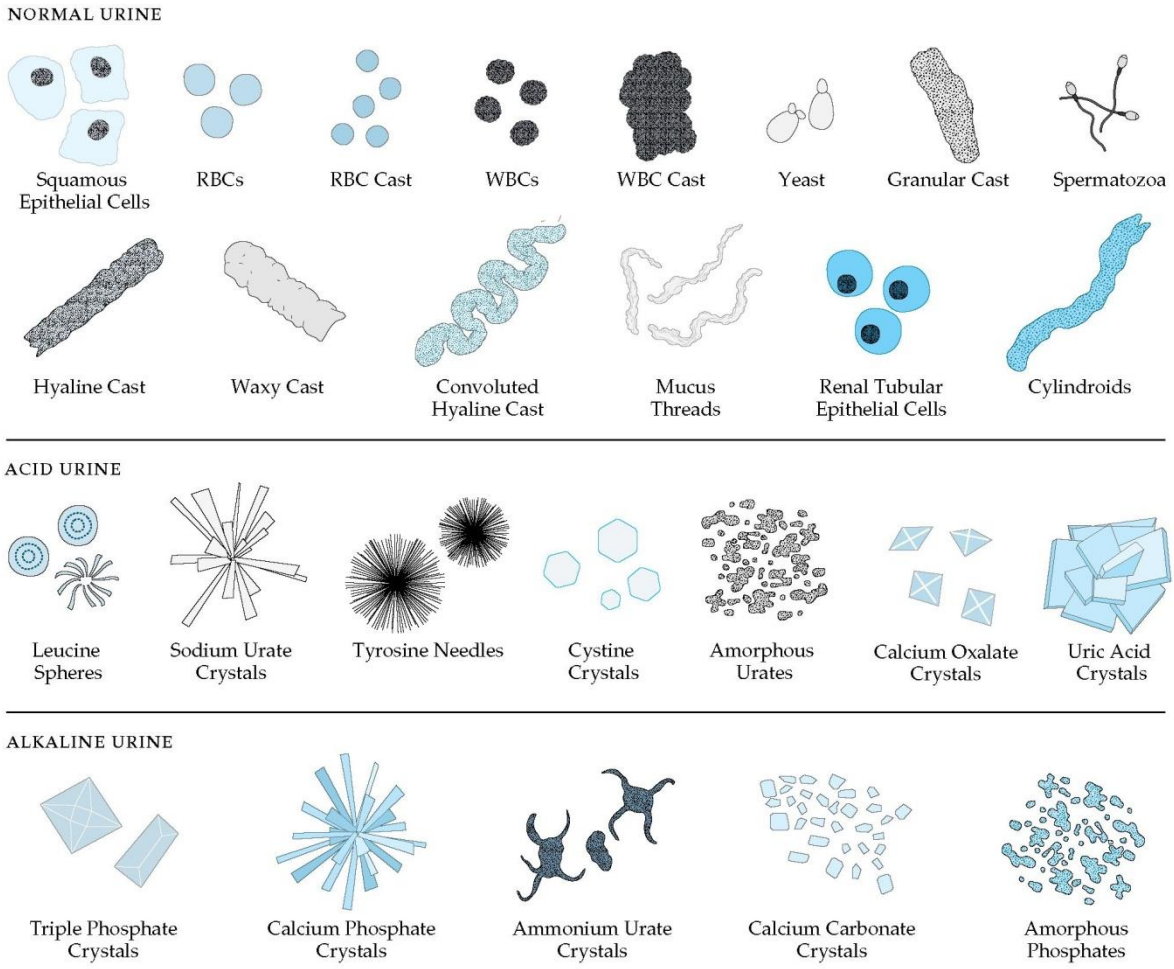
**Types of urinary casts**

1- Acellular casts

- 1- Hyaline casts
- 2- Granular casts
- 3 -Waxy casts
- 4- Fatty casts
- 5- Pigment casts
- 6- Crystal casts

2 Cellular casts

- 1 -Red blood cell casts
- 2- White blood cell casts
- 3 -Bacterial casts
- 4- Epithelial cell casts



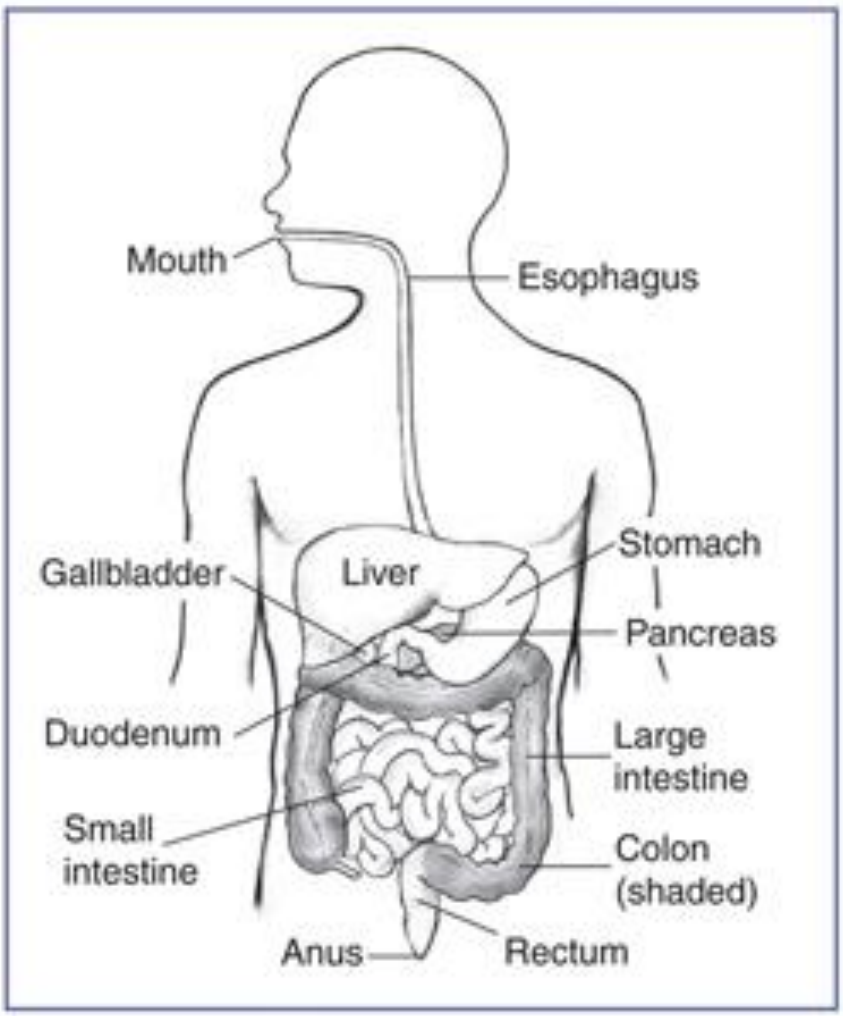

# Electrolytes

**Electrolyte** is a substance that produces an electrically conducting solution when dissolved in a polar solvent, such as water.

**Example**

# General Stool Examination(GSE)

**10**



Stool or faces:-The elimination of digestive waste products from the body is essential to health.

These excreted waste products are known as stool or feces and consisting of.

1. Bile pigments and salts.
2. Intestinal secretions, including mucus.
3. Leukocytes that migrate from the blood stream.
4. Epithelial cells that have been shade.
5. Bacteria and Inorganic material(10-20%) chiefly calcium and
6. phosphates.
7. Undigested and unabsorbed food. • Stool examination is ofte

Types of stool examinations

1. Macroscopical (Physical) examination
2. Fecal Occult Blood (FOB)
3. Microscopical examination
4. Bacteriological examination

**1-Macroscopical examination**

Include :-

1-Stool Consistency

2- Shape

3- Form

4- Amount

5-color

6-Odor

Stool- color

1-Yellow, yellow-green, or green: severe diarrhea

2- Black, usually the result of bleeding in the upper GI tract (>100 mL blood)

3- Maroon, red, or pink: possibly the result of bleeding of the lower GI tract from tumors, hemorrhoids, fissures , or an inflammatory process.

4- Clay colored : biliary obstruction

5- Pale, with a greasy consistency: pancreatic deficiency causing malabsorption of fat.

2. Occult blood test

It is a screening test for digestive carcinoma especially for elderly people. Positive occult blood test is usually due to chronic GI blood loss, both upper and lower GI lesion such as peptic ulcer, stomach carcinoma, and colon carcinoma.

3-Microscopical examination

Wet mount procedure

Materials:

- Microscope slides
- Cover slips
- Normal saline in small bottle with dropper
- Wooden stick
- Fresh stool
- Gloves

## Collection of Fecal Specimens

1-Collect stool in a dry, clean container

2-Uncontaminated with urine or other body secretions, such as menstrual blood

3-Collect the stool with a clean wooden stick.

4-About 20-40 grams of well-formed stool or 5-6 table spoonfuls of watery stool will suffice for a routine examination.

5-Deliver immediately after collection.