Lac. 1

Biomedical sciences are a set of applied sciences that employed natural science knowledge and interventions in healthcare, public health and medicine.

Such disciplines

- Medical microbiology,
- Clinical epidemiology,
- Genetic
- Immunology
- Molecular Biology
- Histopathology
- Hematology
- Clinical Chemistry

Microbiology

- Microbiology is the science that study microorganisms, those being unicellular (single cell), multicellular (cell colony), or a cellular (lacking cells). Microbiology encompasses numerous sub- disciplines including virology, bacteriology, Mycology and phycology.
- **Microbiology have many branches and disciplines such as:**
  1. Medical Microbiology
  2. Food Microbiology
  3. Soil Microbiology
  4. Therapeutic Microbiology.

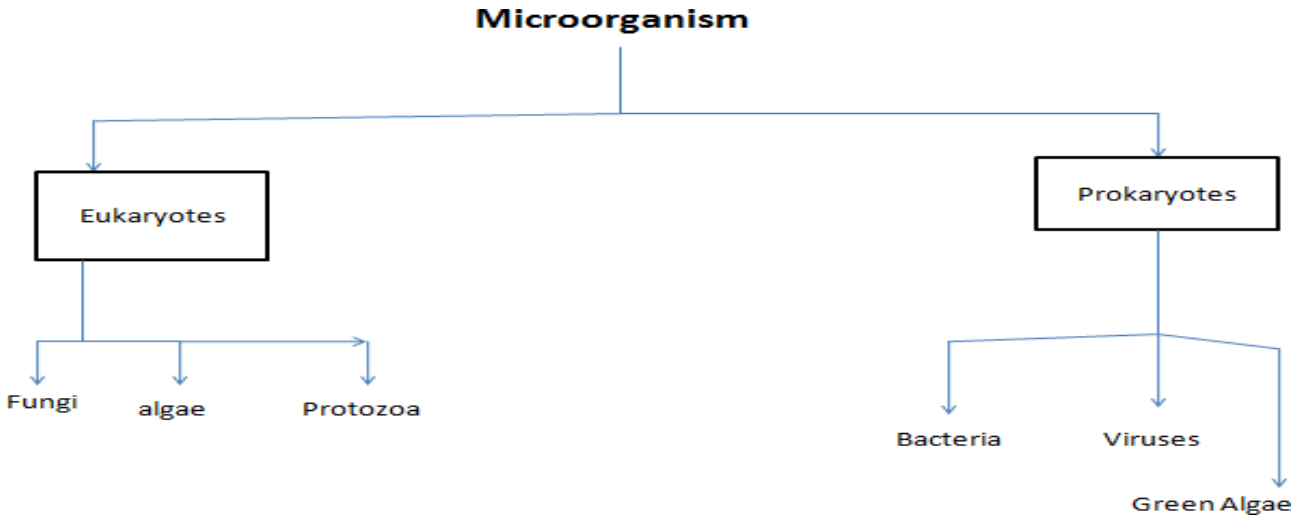
Medical microbiology is a branch of medical science concerned with the prevention, diagnosis and treatment of infectious diseases.

In addition, this field of science studies various clinical applications of microbes for the improvement of health. There are four kinds of microorganisms that cause infectious disease: **bacteria, fungi, parasites and viruses**.

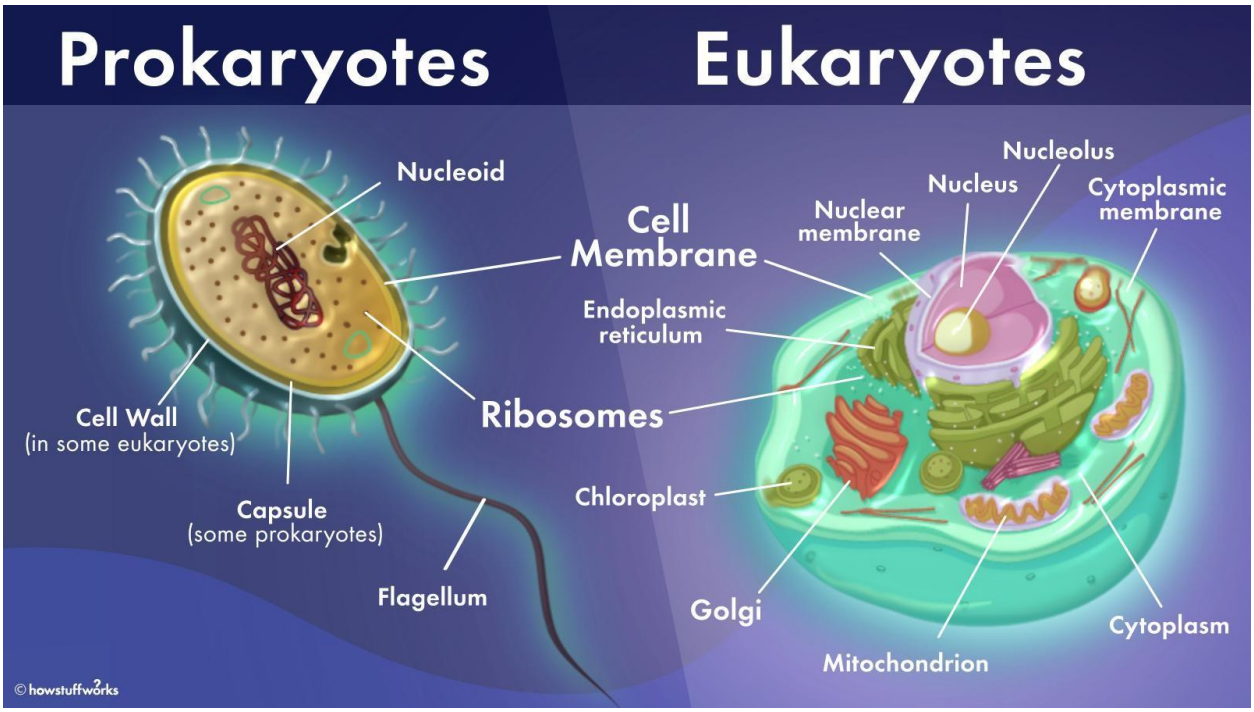
A medical microbiologist studies the characteristics of pathogens, their modes of transmission, mechanisms of infection and growth. Using this information, a treatment can be devised. Medical microbiologists often serve as mentor for physicians, providing identification of pathogens and suggesting treatment options.

Introduction to Microorganisms

1. **Protozoa**: They are small single cell animal belong to the lowest division of the animal kingdom.
2. **Fungi:** these are plants structure devoid from root, stem or leaf. They do not contain chlorophyll and therefore unable to manufacture their own food.
3. **Viruses**: These are organisms which can only multiply within the living cells. They are invisible by optical microscope and not killed by antibiotics.
4. **Bacteria**: are microscopic unicellular free living microorganisms without chlorophyll and having both DNA & RNA and capable of performing all essential processes of life growth, metabolism and reproduction.



No	character	Prokaryotes	Eukaryotes
1	Cell type	Usually unicellular (some cyanobacteria may be multicellular)	Usually multicellular
2	Nucleus, Nucleolus, Nuclear membrane	absent	present
3	DNA & RNA	Present	present
4	Chromosome	One	more
5	Genetic Recombination	Partial, undirectional transfers DNA	Meiosis and fusion of gametes
6	Mitochondria, Lysosomes, Golgi apparatus, Endoplasmic reticulum	absent	present
7	Ribosome	smaller	Larger
8	Cell size	1-10um	10-100um


Prokaryotes
Eukaryotes
Nucleoid
Cell Membrane
Cell Wall
(in some eukaryotes)
Ribosomes
Capsule
(some prokaryotes)
Flagellum
Nucleolus
Nucleus
Cytoplasmic membrane
Nuclear membrane
Endoplasmic reticulum
Chloroplast
Golgi
Cytoplasm
Mitochondrion
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Lac.2

Bacterial cell structure

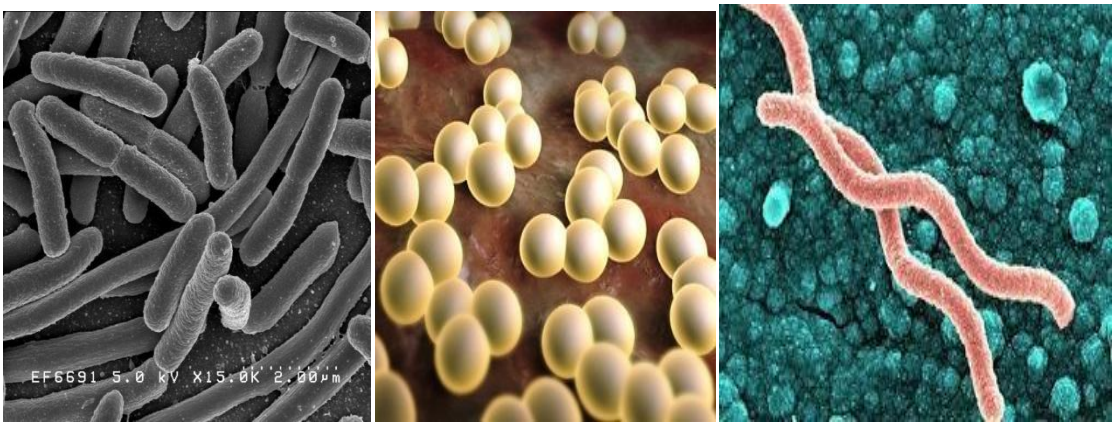
Bacteria, they are simple in structure and contain well developed cell structure which responsible for some of their unique biological structure and pathogenicity. Many structural properties are unique to bacteria and are not found

among archaea or eukaryotes.

Cell morphology

Perhaps the most elemental structural property of bacteria is their morphology (shape). Typical examples include:

- coccus (spherical)
- bacillus (rod-like)
- spiral(DNA-like)
- filamentous (elongated)



bacillus **coccus** **spiral**

Bacterial Cell wall

Cell wall is an important structure of bacteria. It gives **shape**, **rigidity** and

gives **support** to the cell. On the basis of cell wall composition, bacteria are classified into two major group ie. **Gram Positive and gram negative**.

Types of cell wall

1. Gram positive cell wall

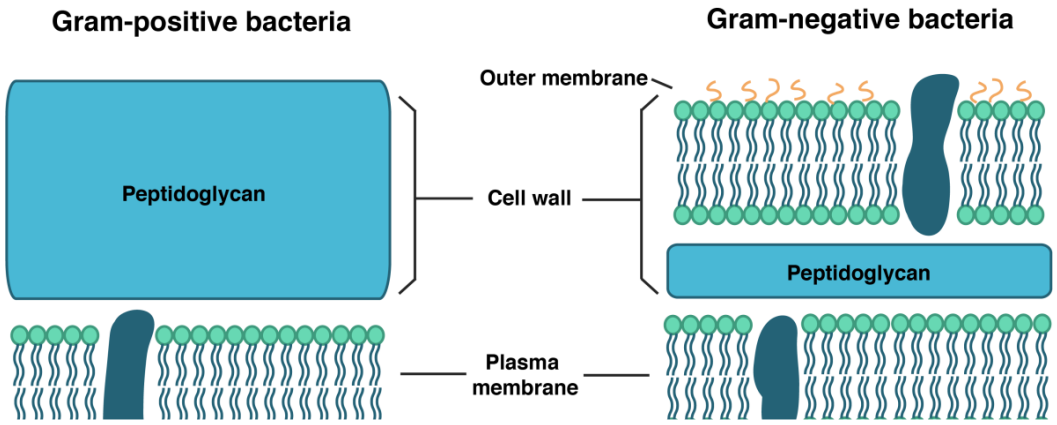
Cell wall composition of gram positive bacteria.

1. Peptidoglycan
2. Lipid
3. Teichoic acid

2. Gram negative cell wall

Cell wall composition of gram negative bacteria

1. Peptidoglycan
2. Outer membrane:
   - Lipid
   - Protein
   - Lipopolysaccharide (LPS)



Classification of Media

- **Nutrient media can be subdivided:**

1. Simple media - meat-peptone broth (MPB), meat-peptone agar (MPA)
2. Synthetic media
3. Complex media
4. Special media: a) Enriched media; b) Enrichment media; c) Selective media; d) Indicator and differential media; e) Sugar media; f) Transport media.

The basic requirements of culture media

- ***energy source;***
- ***carbon source;***
- ***nitrogen source;***
- ***salts like sulphates, phosphates, chlorides and carbonates of sodium, potassium, magnesium, ferric, calcium and trace elements, like copper, etc.;***
- ***satisfactory pH 7.2-7.6;***
- ***growth factor like vitamins***

***Classification of Media On the basic of consistency*:**

- Solid media
- Liquid media
- Semisolid media

Sterilization

Sterilization (or **sterilisation**) refers to any process that eliminates, removes, kills, or deactivates all forms of life and other biological agents (such as fungi, bacteria, viruses, spore forms, prions, unicellular eukaryotic organisms such as Plasmodium, etc.)

TREAT-MENT	***TEMP E-RATU RE***	***EFFECTIVENESS***
Incineration	>500 C	Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process.
Boiling	100 C	Thirty minutes of boiling kills vegetative forms of bacteria but may not kill bacterial endospores. There are also toxins that are not inactivated at 100C.
Intermittent boiling	100 C	Three 30-minute intervals of boiling, followed by periods of cooling kills bacterial endospores.
Autoclave (steam under pressure)	121 C for 15 minutes at 15 p.s.i.	Kills all forms of life including bacterial endospores. The substance being sterilized must be maintained at the effective temperature for the entire time.
Dry heat (hot air oven)	160 C for 2 hours	Used for materials that must remain dry. Good for glassware, metal, but not most plastic or rubber items.

Dry heat (hot air oven)	170 C for 1 hour	Same as above. Note that increasing the temperature by 10 C shortens the sterilizing time by 50 %.
Pasteurization (batch method)	63-66 C for 30 minutes	Kills most vegetative bacterial cells, including pathogens such as streptococci, staphylococci and Mycobacterium tuberculosis.
Pasteurization (flash method)	72 C for 15 seconds	Effect on bacterial cells is similar to batch method. For milk, this method has fewer undesirable effects on quality or taste.

Lac.3

ISOLATION & IDENTIFICATION OF BACTERIA

IDENTIFICATION METHODS

The most important task for bacteriologist is to identify the pathogens from the clinical sample so that appropriate treatment can be provided.

There are several methods to identify different type of bacteria.

1. Isolation in pure form

2. Staining reaction

3. Morphology of bacterial colony

4. Metabolism

5. Biochemical property

1. Isolation in pure form

Studies on the biochemical, antigenic and other characters of bacteria can be done only if the organism available in the pure form.

Technique:

a. Plating on solid culture media, clinical sample is streaked onto a solid medium (like: MacConkey agar, nutrient agar or blood agar) in such a way so as to ensure isolated discrete colonies.

b. Use of selective growth condition, most important example of this is the growth of anaerobic bacteria which will not take place in an environment having oxygen.

2. Staining reaction

a. The age of the culture is important. In older cultures, staining characteristics either vary or are not brought out well. Simple stains bring out the best morphology. Differential and special stains are necessary to bring out characteristics like: gram negative and gram positive bacteria,

Acid fast and non acid fast , spirochetes, capsule and Flagella, etc

a. **Gram stain**

Gram stain divides the bacteria into Gram positive & Gram negative. The basic procedure goes like this:

i. Take a heat fixed bacterial smear.
ii. Flood the smear with CRYSTAL VIOLETfor 1 minute, then wash with water. [PRIMARY STAIN]
iii. Flood the smear with IODINE for 1 minute, then wash with Water.
iv. Flood the smear with ETHANOL.ACETONE, quickly, then wash with water. [DECOLORI
v. Flood the smear with SAFRANIN for 1 minute, then wash with water. [COUNTERSTAIN]
vi. Blot the smear, air dry and observe.

..Examine under microscope

i. Gram positive bacteria. violet

ii. Gram negative bacteria. Pink

Shape of Bacteria

..Bacteria display three basic shapes:

i. cocci
ii. rod shaped –bacilli
iii. spiral.

b. **Ziehl -Neelsen Staining**

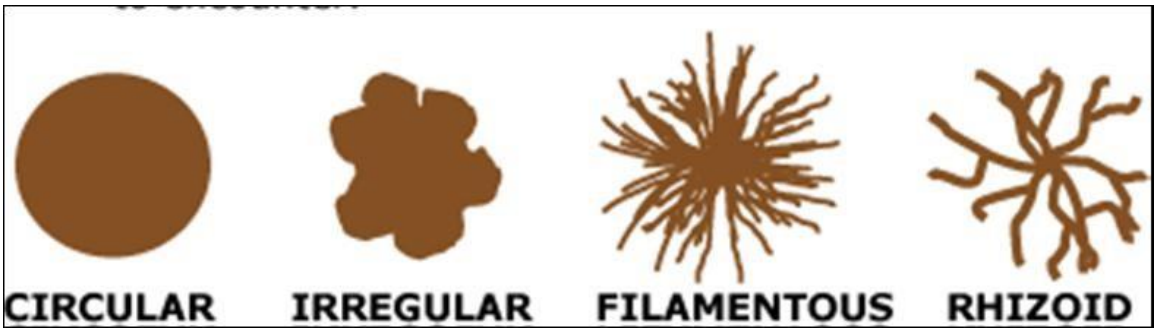
The **Ziehl–Neelsen stain**, also known as the **acid-fast stain**, It is a special bacteriological stain used to identify acid-fast organisms,
mainly Mycobacteria. *Mycobacterium tuberculosis* is the most important of this group because it is responsible for tuberculosis (TB).

c. **India ink (capsule stain)**

The capsule stain employs an acidic stain and a basic stain to detect capsule production. Capsules are formed by organisms such as Klebsiella pneumoniae. Most capsules are composed of polysaccharides, but some are composed of polypeptides

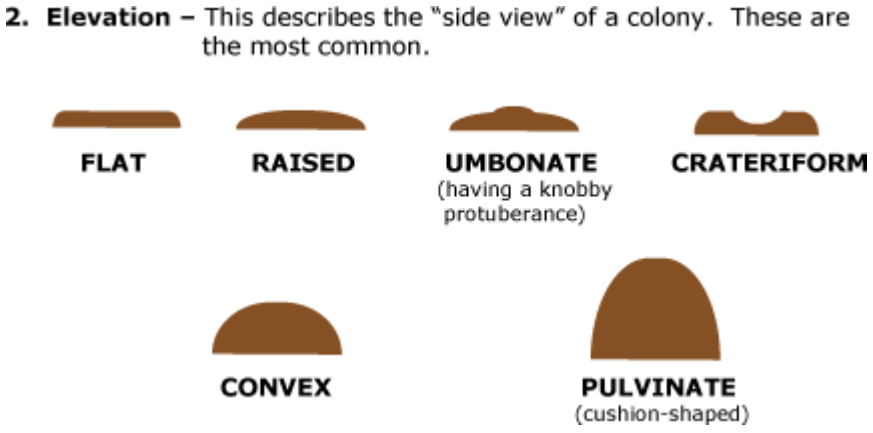
3. Morphology of the bacterial colony:

i. Shape: circular, irregular, radiate or rhizoid.



ii. Size: diameter in mm

iv. Elevation: flat, raised, low convex, dome shaped



iv. Margin: Entire, wavy, lobate, filiform

3. Margin – The margin or edge of a colony (or any growth) may be an important characterisic in identifying an organisms. Several examples are shown below.

v. Surface: smooth, wavy, rough, granular, papillate, glistening etc.

IN A FLUID MEDIUM FOLLOWING CHARACTERS ARE OBSERVED

i. Degree of growth-Absence, scanty, moderate, abundant etc.
ii. Present of turbidity and its nature.
iii. Presence of deposit and its character.
iv. Nature of surface growth.
iv. Ease and disintegration and odor.

5. METABOLISM

To classify the differentiate species following aspects are studied

i. Requirement of oxygen

ii. The need of co2

iii. Capacity to form pigments

iv. Power of hemolysis

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5. **Biochemical Tests:**
   - Case Study Tests
   1. Methyl Red/Voges Proskauer
   2. Citrate
   3. H2S production in SIM
   4. Motility
   5. Lactose fermentation
   6. Sucrose fermentation
   7. Glucose fermentation & gas production
   8. Triple Sugar Iron Agar (TSI) test
   9. Indole

Lac.4

STAINING

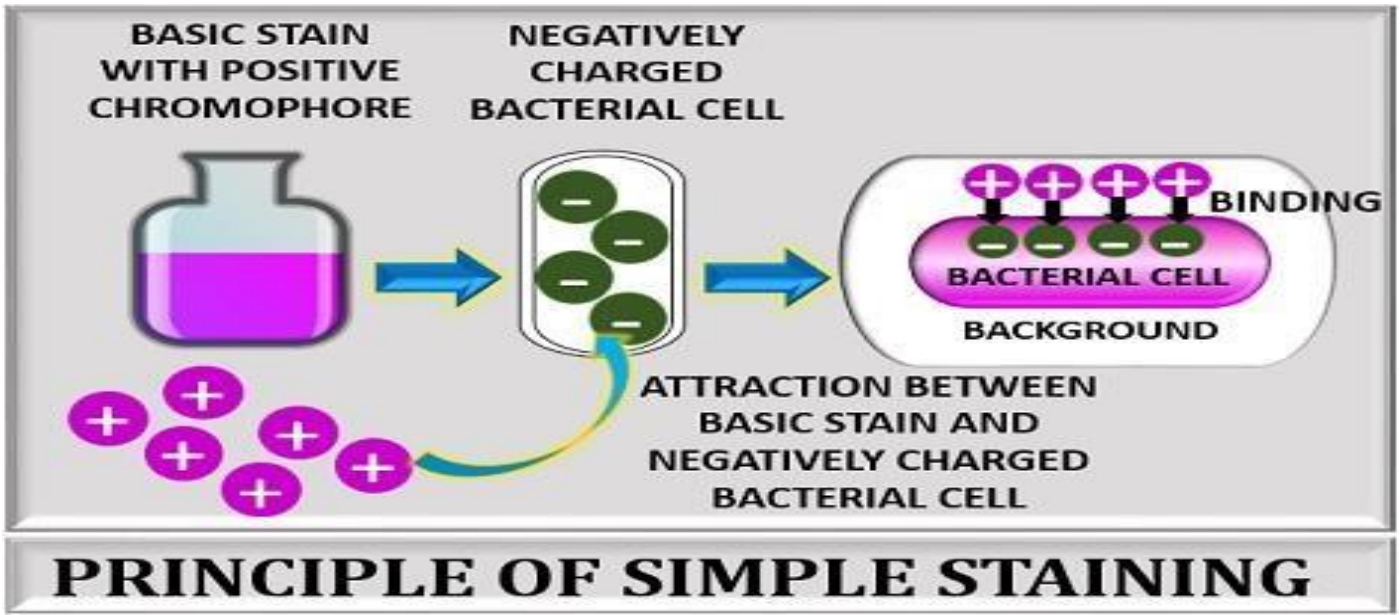
Staining :- is a technique used to enhance contrast in samples, generally at the microscopic level and divided to types

In vivo staining (also called vital staining) is the process of dyeing living tissues.

In vitro staining involves coloring cells or structures that have been removed from their biological context.

Stains (dyes):-

Stains are generally salts in which one of the ions is colored. (A salt is a compound composed of a positively charged ion and a negatively charged ion.) For example,



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slight negative charge when growing in a medium of near neutral pH and will therefore attract and bind with basic dyes. Some examples of basic dyes are crystal violet, safranin, basic fuchsin and methylene blue.

B- Acid dyes have negatively charged chromophores and are repelled by the bacterial surface forming a deposit around the organism. They stain the background and leave the microbe transparent. Nigrosine red are examples of acid dyes.

There are several staining methods that are used routinely with bacteria.

1-Simple stain: - Simple staining techniques that enable microbiologists to observe the morphological characteristics of bacteria. Although simple stains are useful, they do not reveal details about the bacteria other than morphology and arrangement.

2- DIFFERENTIAL STAINS

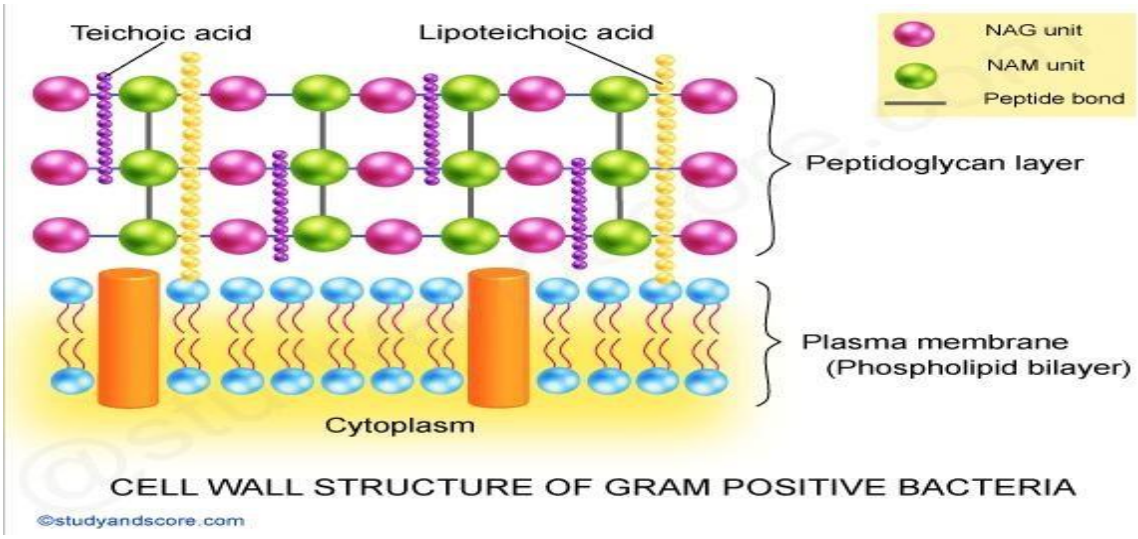
Differential stains give varying results depending on the organism being treated. These results are often helpful in identifying the microbe

A. Gram Stain B. Acid-fast Stain

A- Gram stain :- The Gram stain is a differential stain commonly used in the microbiology laboratory that differentiates bacteria on the basis of their cell wall structure. Most bacteria can be divided into two groups based on the composition of their cell wall:-

1) Gram-positive cell walls have a **thick peptidoglycan layer** beyond the plasma membrane.

Characteristic polymers called teichoic and lipoteichoic acids stick out above the **peptidoglycan** and it is because of their negative charge that the cell wall is overall negative. These acids are also very important in the body's ability to recognize foreign bacteria. Gram-positive cell walls stain blue/purple with the Gram stain.



2) **Gram-negative** cell walls are more complex. They have a thin peptidoglycan layer and an outer membrane outside the plasma membrane. The space between the plasma membrane and the outer membrane is called the periplasmic space. The outer leaflet of the outer membrane is composed largely of a molecule called **lipopolysaccharide (LPS). LPS** is an endotoxin that is important in triggering the body's immune response and contributing to the overall negative charge of the cell. across the outer membrane are porin proteins that enable the passage of small

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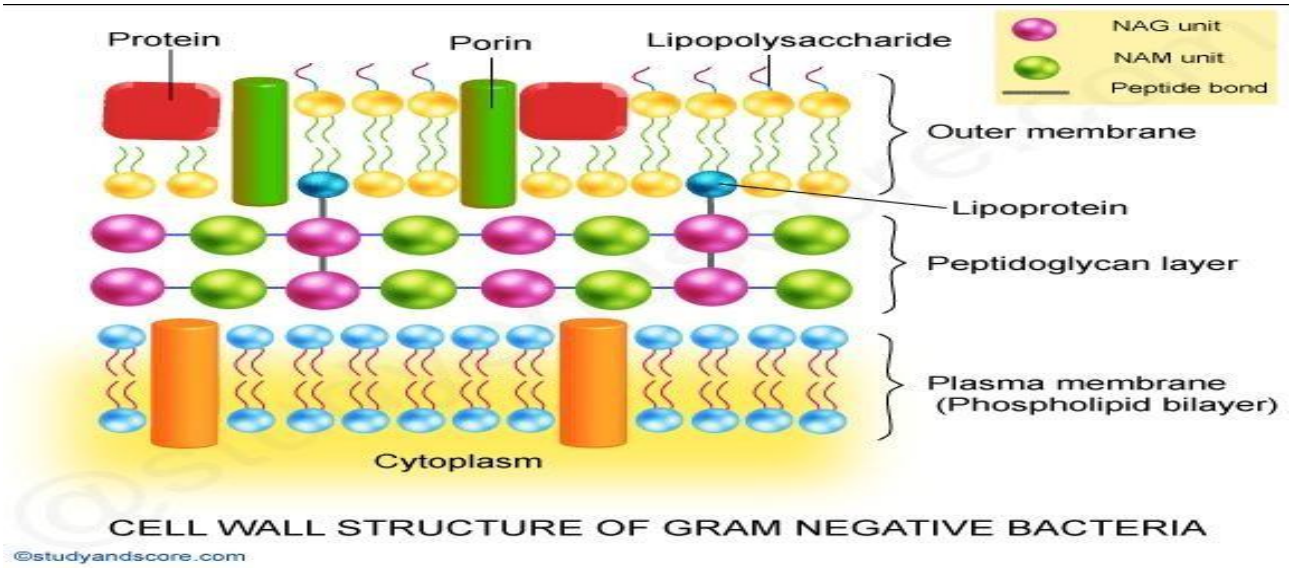


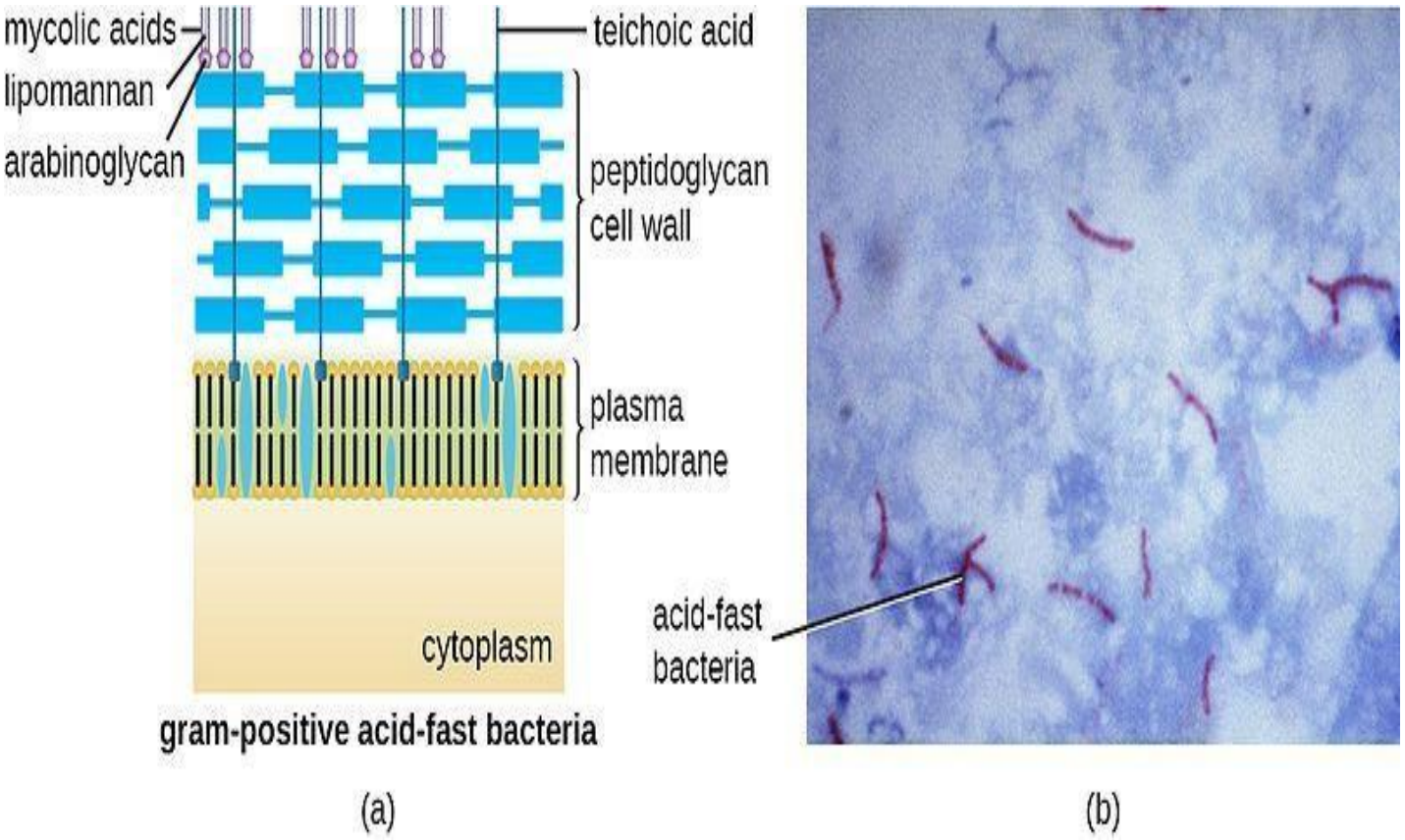
CELL WALL STRUCTURE OF GRAM NEGATIVE BACTERIA

molecules. Lipoproteins join the outer membrane and the thin peptidoglycan layer. Gram-negative cells will stain pink with the Gram stain.

B. Acid-fast Stain (Ziehl Neelsen Acid-fast stain):-

Mycobacterium and many Nocardia species are called acid-fast because during an acid-fast staining procedure they retain the primary dye carbol fuchsin despite decolorization with the powerful solvent acid-alcohol (95% ethanol with 3% HCl). Nearly all other genera of bacteria are nonacid-fast.

The acid fast genera have the waxy hydroxy-lipid called **mycolic acid** in their cell walls. It is assumed that mycolic acid prevents acid-alcohol from decolorizing protoplasm.



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Bacterial growth

Bacterial growth is the asexual reproduction, or cell division, of a bacterium into two daughter cells, in a process called binary fission. Providing no mutational event occurs, the resulting daughter cells are genetically identical to the original cell.

Phases

1. During **lag phase**, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant.

2. The **log phase** (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day. Exponential growth cannot continue indefinitely, however, **because**

the medium is soon depleted of nutrients and enriched with wastes.

3. The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. **Stationary phase results from a situation in which growth rate and death rate are equal**. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary phase. Recent studies, presented evidence that DNA damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria.

4. At **death phase** (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

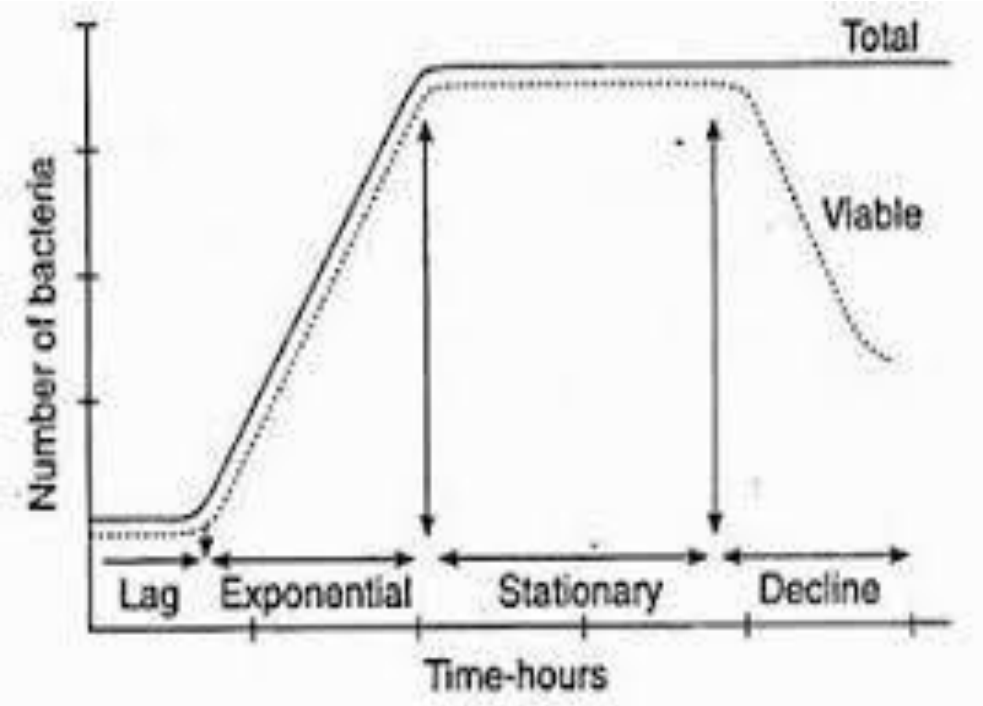


Fig. 2.18 : Bacterial growth curve

Lac.6

The Blood

Blood is a bodily fluid in humans that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. Blood is a type of connective tissue that consists of blood cells suspended in liquid matrix (blood plasma), contains antibodies, nutrients, oxygen and much more to help the body work.

Functions

Blood performs many important functions within the body including:

1. Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells).
2. Supply of nutrients such as glucose, amino acids and fatty acids (dissolved in the blood or bound to plasma proteins).
3. Removal of waste such as carbon dioxide, urea and lactic acid.
4. Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies.
5. Messenger functions, including the transport of hormones and the signaling.
6. Regulation functions, including regulation of body pH, temperature and hydraulic.

The Blood Plasma

Plasma is a pale yellow fluid have total volume of (2.7–3.0) liters, It is mostly **water (92%** by volume) and contains **proteins, glucose, ions, hormones and carbon dioxide. Plasma proteins** include:

• **Albumin** makes up 60% of the plasma proteins, and it functions to **regulate the colloidal osmotic pressure of blood.**

• **Globulins** account for 36% of the plasma proteins, some of it are part of the

immune system, **whereas others function as transport molecules**.

• **Fibrinogen** constitutes 4% of plasma proteins and **responsible for the formation of blood clots.**

Plasma circulates dissolved nutrients, such as glucose, amino acids and fatty acids, and removes waste products, such as carbon dioxide, urea and lactic acid.

The term serum refers to plasma from which the clotting proteins have been removed. Most of the proteins remaining are albumin and immunoglobulins.

The Blood Cells

The blood cells are mainly red blood cells (also called RBCs or erythrocytes), white blood cells (also called WBCs or leukocytes) and platelets (also thrombocytes).

❖ **Red Blood Cells**

The red blood cells are disk-shaped cells with edge thicker than center of the cell, contain hemoglobin that give it red color. The erythrocytes are (4.7-6.1 million) cell/mm3 in male, (4.2-5.4 million) cell/mm3 in female, constitute about 45% of whole blood. The primary functions of erythrocytes are **to transport oxygen from the lungs to the various tissues of the body and to assist the transport of carbon dioxide from the tissues to the lungs.** Mature red blood cells lack a nucleus and organelles, and unable to divide, live for about 120 days.

❖ **White Blood Cells**

White blood cells are spherical cells that are whitish in color. They are larger than erythrocytes; they have a nucleus, and can leave the blood and move by amoeboid Movement through the tissues .

Leukocytes are (4,000–11,000) cell/ mm3, constitute about 0.7% of whole blood, consider a part of the body's immune system; they **destroy and remove old or abnormal cells and cellular waste, as well as attack infectious agents**

(pathogens) and foreign substances. Leukocytes are named according to their appearance:

1- Granulocytes: containing large cytoplasmic granules. There are three kinds:

• **Neutrophils** are the most common type of leukocytes. They phagocytes foreign substances.

• **Basophils** are release histamine and other chemicals that promote inflammation, and heparin that prevents the formation of clots.

• **Eosinophils** release chemicals that reduce inflammation.

2-Agranulocytes: containing very small cytoplasmic granules. There are two kinds:

• **Lymphocytes** play an important role in the body's immune response by production of antibodies and other chemicals.

• **Monocytes** become macrophages after they leave the blood and enter tissues to ingest bacteria, dead cells and any other debris within the tissues.

❖ Platelets

Platelets are minute fragments of cells, consisting of a small amount of cytoplasm surrounded by a cell membrane. They are (200,000–500,000) **thrombocytes,** take part in blood clotting (coagulation) and preventing blood loss.

Hemoglobin

Hemoglobin is an iron-containing protein in the blood, responsible for oxygen transport and determinant of the blood color. Each molecule of hemoglobin consists of four protein chains bound to four heme groups. Each heme contains one iron atom, which is necessary for the normal function of hemoglobin, where is bind to oxygen molecule.

Hemoglobin interaction with various molecules alters the exact color. Arterial and capillary bloods are bright red when its hemoglobin is oxygenated and dark red in veins when it is deoxygenated.

Lac.7

Production and degradation of blood cells

The various cells of blood are made in the bone marrow in a process called **hematopoiesis**, which includes

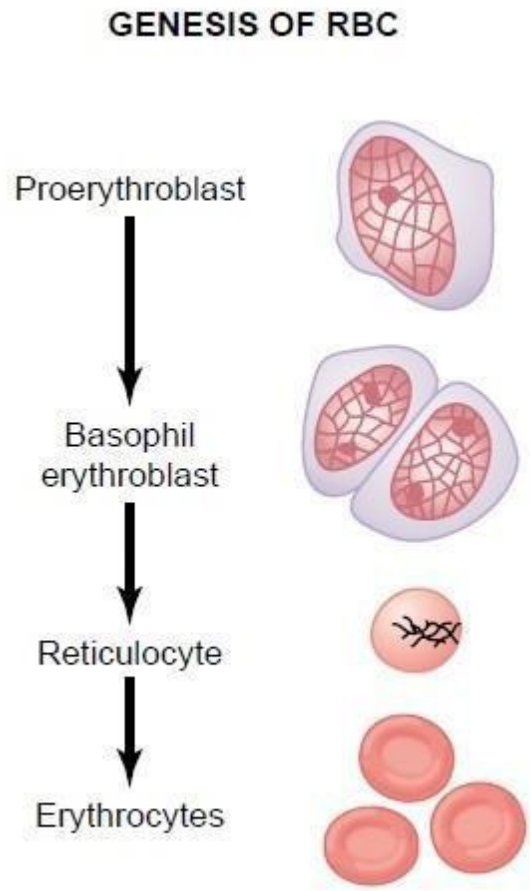
Erythropoiesis, the production of red blood cells;

myelopoiesis, the production of white blood cells and platelets.

In the fetus, **hematopoiesis** occurs in several tissues such as the **liver, thymus gland, spleen, lymph nodes and red bone marrow.**

Erythropoiesis is the process which produces red blood cells (erythrocytes). It is stimulated by decreased O2 in circulation. The proliferation and differentiation of red cell precursors stimulates by **erythropoietin**, which activates increased erythropoiesis in the hemopoietic tissues, ultimately producing red blood cells.

The process of erythrocytes production requires vitamin B12, folic acid and iron. Thus lack of these can interfere with normal erythrocytes production. Healthy erythrocytes live for about 120 days before they are degraded by the spleen and liver. Hemoglobin is broken down, iron and amino acids are reused and heme becomes bilirubin that is secreted in bile.



Blood Clotting

Blood clotting or coagulation is formation of a clot, which is a network of thread like protein fibers, called fibrin. The formation of a blood clot depends on a number of proteins found within plasma called clotting factors. There are three steps in the clotting process:

1. Activation of clotting factors by exposure to connective tissues and chemicals, resulting in the formation of prothrombin activator (**prothrombinase**).

2. Conversion of prothrombin to **thrombin** by prothrombinase.

3. Conversion of fibrinogen to **fibrin** by thrombin. The fibrin fibers form a network, which traps blood cells and platelets to form the clot.

Rupture or damage of the blood vessels

↓

Activation of clotting factors

↓

Formation of *prothrombin activator* → ***Prothrombin***

↓ Ca^{++}

Thrombin

Fibrinogen → Fibrin

Ca^{++}

Blood cells & platelets → Network of fibrin fibers

Blood clotting

Most of the clotting factors are manufactured in the liver, and many of them require vitamin K for their synthesis. In addition, many of the chemical reactions of clotting require calcium ions and chemical release from platelets. Low levels of vitamin K, Low levels of calcium, low numbers of platelets, or liver dysfunction can seriously impair the blood clotting process.

Blood Grouping

In human, blood is categorized by the ABO blood group system. Blood groups are determined by antigens on the surface of erythrocytes. Type A blood has A antigens, type B blood has B antigens, type AB blood has A and B antigens, and type O blood does not have A or B antigens.

In addition, in the plasma there are proteins called antibodies. Type A blood has B antibodies, type B blood has A antibodies, type AB blood does not have A or B antibodies, and type O blood has A and B antibodies.

When the antibodies bind to the antigens, they form molecular bridges that connect the erythrocytes together, and **agglutination** or clumping of cells occurs. This combination also can initiate reactions that cause hemolysis or rupture of the erythrocytes. It is known as transfusion reactions, are caused by interactions between antigens and antibodies.

Usually a **donor** (person who gives blood) can give blood to a **recipient** (person who receives blood) if they both have the same blood type. Historically, people with type **O blood** have been called **universal donors** because they usually can give blood to the other ABO blood types without causing an ABO transfusion reaction. People with type **AB blood** were called **universal recipients** because they could receive type A, B, AB or O blood with little probability of a transfusion reaction.

Rh Blood Groups

Another important blood group is the Rh blood group, so named because it was first studied in the rhesus monkey. Rh-positive people have certain Rh antigens on the surface of erythrocytes, and they Rh-negative don't have these Rh antigens. About 85% of people are Rh-positive.

Bleeding time

- **Bleeding time** is a medical test done on someone to assess their platelets function. It involves making a patient bleed then timing how long it takes for them to stop bleeding.
- The bleeding time test is old method and indicated when other more reliable and less invasive tests for determining coagulation are not available.
- **Abnormal results could also indicate the following conditions:**
    1. A **blood vessel defect** is any condition that affects how well your blood vessels transport blood through your body.
    2. A **genetic platelet function defect** is a condition present at birth that affects how well your platelets function. Hemophilia is one example of this type of defect.
    3. **Primary thrombocythemia** is a condition in which your bone marrow creates too many platelets.
    4. **Thrombocytopenia** is a condition that causes your body to produce too few platelets.
    5. **Von Willebrand's disease** is a hereditary condition that affects how your blood coagulates, or clots.

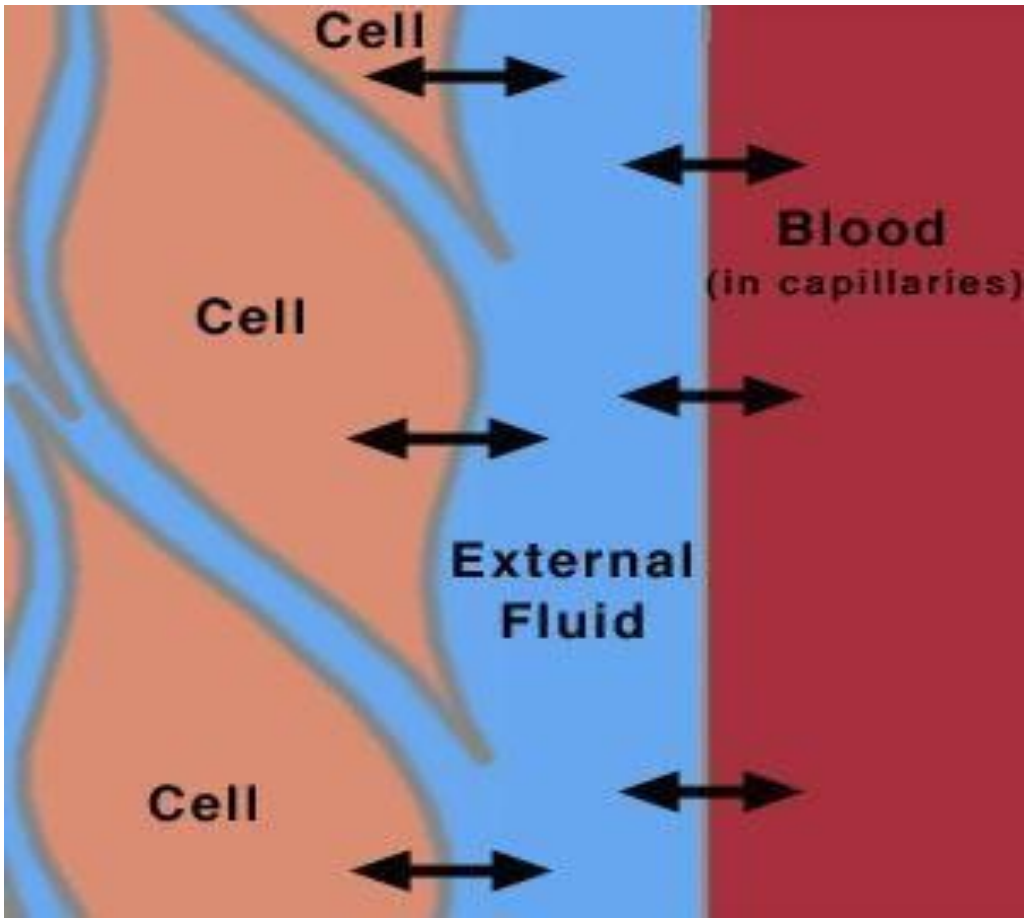
- Normal bleeding time is between **1 and 8 minutes**. Results outside of that range could indicate a platelet defect and will require further testing.

- **Clotting time** is the time required for a sample of blood to coagulate in vitro under standard conditions.
- There are various methods for determining the clotting time, the most common is the capillary tube method.
- Normal value of clotting time is **8 to 15 minutes**.
- Activated partial thromboplastin time (aPTT) is used for heparin studies and the normal range is 20–36 seconds, depending upon which type of activator is used in the study.
- APTT is a basic coagulation screening test, it is useful tool for to intrinsic coagulation pathway
- Prothrombin time (PT) is used for warfarin studies and the normal values differ for men and women. PT time for adult males' normal range is 9.6-11.8 seconds, while adult females' normal range is 9.5-11.3 seconds
- PT is a basic coagulation screening test, it is useful tool for to extrinsic coagulation pathway

Lac.9

Body Fluids

- Total amount of fluid in the human body is approximately 70% of body weight
- Body fluid has been divided into two compartments –
  - **Intracellular fluid (ICF)**
    - Inside the cells
    - 55% of total body water
  - **Extracellular fluid (ECF)**
    - Outside the cells
    - 45% of total body water


Cell
Blood
(in capillaries)
Cell
External
Fluid
Cell

Body Fluid Compartments

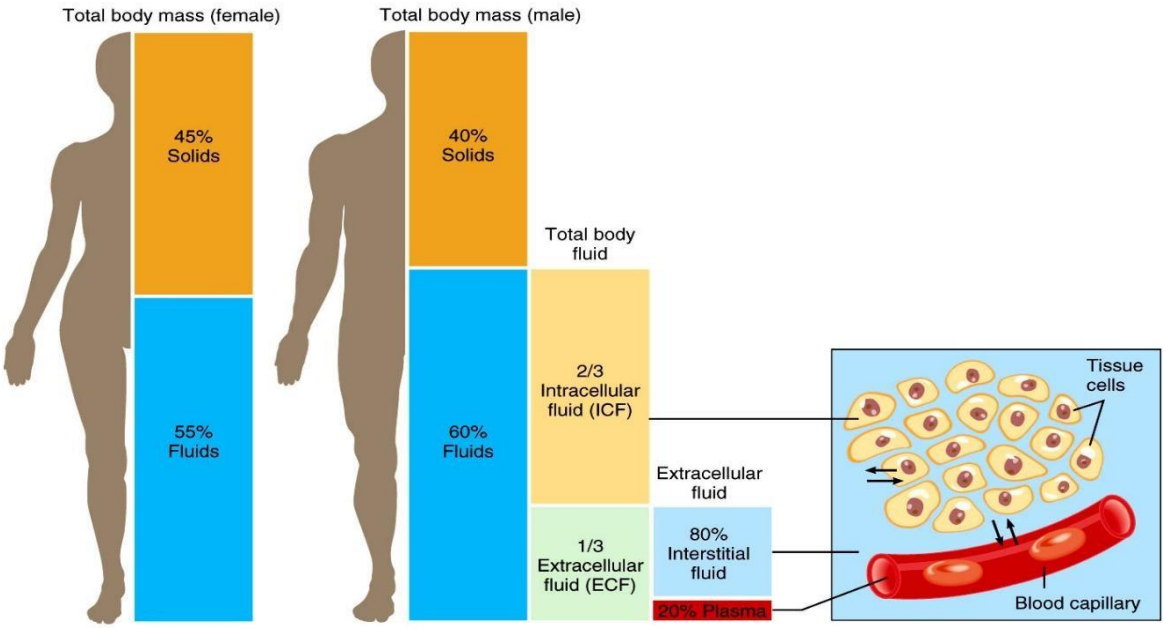


Figure 27.01 Tortora - PAP 12/e

Composition of body fluids

Organic substances	Inorganic substances
Glucose	Sodium
Amino acids	Potassium
Fatty acids	Calcium
Hormones	Magnesium
Enzymes	Chloride
	Phosphate

Extracellular fluid

- **Interstitial fluid**
  - Present between the cells
  - Approximately 80% of ECF
- **Plasma**
  - Present in blood
  - Approximately 20% of ECF
- **Also includes**
  - Lymph
  - synovial fluid
  - cerebrospinal fluid

- 1) **Extracellular fluids**:
- *a)* ***Interstitial Fluid****:* also known as intercellular fluid and tissue fluid is fluid between the cells of multi-cellular organisms which delivers materials to the cells, intercellular communication, and removal of metabolic waste.

- it represents the largest portion of the ECF compartment.
- Interstitial fluid consists of a water solvent containing amino acids, sugars, fatty acids, coenzymes, hormones, neurotransmitters, salts, as well as waste products from the cells.
- This fluid presents as gel-like extracellular
- The plasma and the interstitial fluid integrate through pores in the blood capillaries which allow water and most dissolved substances except protein to diffuse .

Barriers separate ICF, interstitial fluid and plasma

- Plasma membrane :Separates ICF from surrounding interstitial fluid
- Blood vessel wall :Separate interstitial fluid from plasma

*2)**Blood plasma:***

It is the fluid portion of the blood.

- The blood transports oxygen from the lungs to the body cells and carbon dioxide from the body cells to the lungs.

- Blood also transports nutrients derived from food in the intestine to the body cells., other nutrients between organs

3) **Lymph**

- Clear and colorless fluid
- 96% water and 4% solids
- Solids –
  - **Proteins**
    - 2-6% of solids
    - albumin, globulin, fibrinogen, prothrombin, clotting factors, antibodies, enzymes
  - **Lipids**
    - 5-15%
    - Chylomicrons
    - Lipoproteins
  - **Carbohydrates**
    - Glucose mainly
  - NPN (Non protein Nitrogen)
    - Urea and creatinine

- **Electrolytes**
  - Sodium, calcium, potassium, chloride, bicarbonates

Functions of Lymph

- Return protein from tissue spaces into blood
- Removal of bacteria, toxins and other foreign bodies from tissues
- Maintain structural and functional integrity of tissue
- Route for intestinal fat absorption
- Transport lymphocytes

2)Intracellular Fluid:

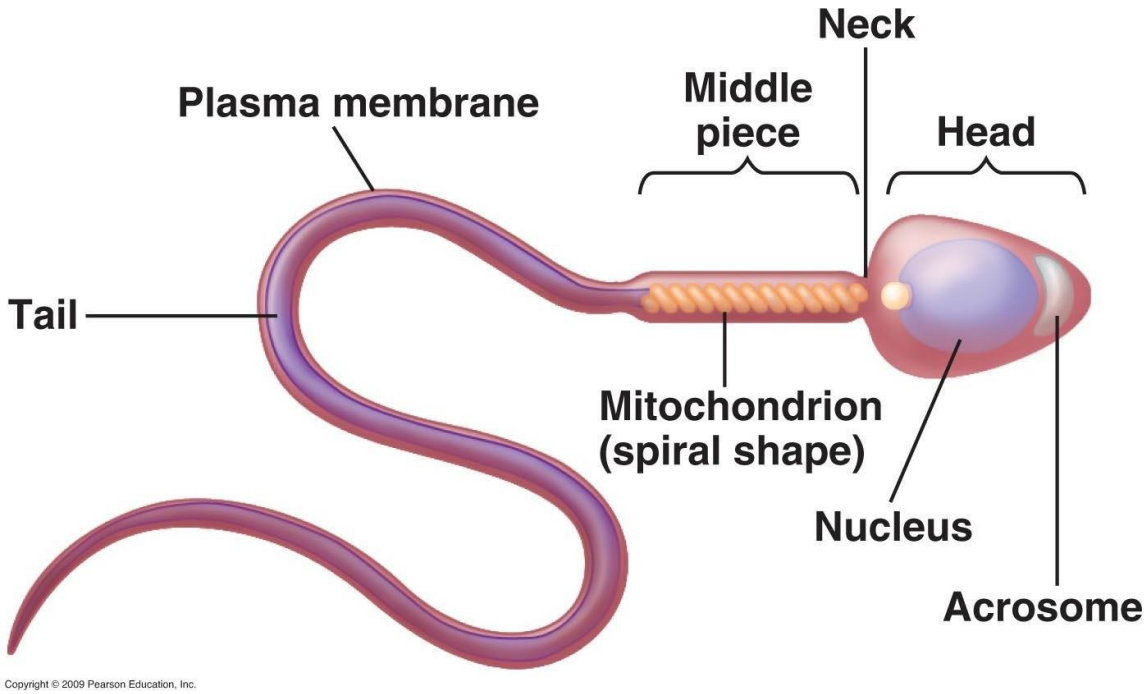
- The cytosol or intracellular fluid is the liquid found inside the cells .
- Physiological Function :
- The cytosol has no single function and instead it is the site of multiple cell processes including metabolic processes (such as glycolysis, gluconeogenesis) . It is also involved in signal transduction from the cell membrane to sites within the cell .

Lec.10

Sperm

Sperm is the male reproductive cell and is derived from the Greek word *sperma* (meaning "seed"). In the types of sexual reproduction known as anisogamy and its subtype oogamy, there is a marked difference in the size of the gametes with the smaller one being termed the "male" or sperm cell. A uniflagellar sperm cell that is motile is referred to as a **spermatozoon**, whereas a non-motile sperm cell is referred to as a **spermatium**. Sperm cells cannot divide and have a limited life span, but after fusion with egg cells during fertilization, a new organism begins developing, starting as a totipotent zygote. The human sperm cell is haploid,

so that its 23 chromosomes can join the 23 chromosomes of the female egg to form a diploid cell. In mammals, sperm develops in the testicles and is released from the penis.



Semen Analysis

Semen analysis, also known as a sperm count test, analyzes the health and viability of a man's sperm. Semen is the fluid containing sperm (plus other sugar and protein substances)
that's released during male ejaculation. A semen analysis measures three major factors of sperm health:

- the number of sperm
- the shape of the sperm
- the movement of the sperm, also known as "sperm motility"

Sample collection instructions:

- avoid ejaculation for 24 to 72 hours before the test
- avoid alcohol, caffeine, and drugs such as cocaine and marijuana two to five days before the test
- stop taking any herbal medications
- avoid any hormone medications

Sperm morphology

Sperm morphology — the size and shape of sperm — is one factor that's examined as part of a semen analysis to evaluate male infertility. Sperm morphology results are reported as the percentage of sperm that appear normal when semen is viewed under a microscope.

Normal sperm have an oval head with a long tail. Abnormal sperm have head or tail defects — such as a large or misshapen head or a crooked or double tail. These defects might affect the ability of the sperm to reach and penetrate an egg

Volume

According to one lab test manual semen volumes between 1.5mL and 5 mL are normal. Low volume may indicate partial or complete blockage of the seminal vesicles, or that the man was born without seminal vesicles. In clinical practice, a volume of less than 1.5 mL in the setting of infertility and absent sperm should prompt an evaluation for obstructive azoospermia.

Color

Semen normally has a whitish color. It tends to get a yellowish tint as a man ages. Semen color is also influenced by the food we eat: foods that are high in sulfur, such as garlic, may result in a man producing yellow semen. Presence of blood in semen (hematospermia) leads to a brownish or red colored ejaculate. Hematospermia is a rare condition.

Semen that has a deep yellow color or is greenish in appearance may be due to medication. Brown semen is mainly a result of infection and inflammation of the prostate gland, urethra and seminal vesicles. Other causes of unusual semen color include sexually transmitted infections such as gonorrhea and chlamydia, genital surgery and injury to the male sex organs.

Fructose level

Fructose level in the semen may be analyzed to determine the amount of energy available to the semen for moving. WHO specifies a normal level of 13 μmol per sample. Absence of fructose may indicate a problem with the seminal vesicles.

pH

According to WHO criteria specify normal PH as 7.2-7.8. Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. A basic ejaculate (higher pH value) may indicate an infection. A pH value outside of the normal range is harmful to sperm and affect their ability to penetrate the egg.

Liquefaction

The liquefaction is the process when the gel formed by proteins from the seminal vesicles is broken up and the semen becomes more liquid. It normally takes less than 20 minutes for the sample to change from a thick gel into a liquid.

Sperm motility

Sperm motility describes the ability of sperm to move properly through the female reproductive tract (internal fertilization) or through water (external fertilization) to reach the egg.

A man can have a total number of sperm far over the limit of 20 million sperm cells per milliliter, but still have bad **quality because too few of them are motile**. **A more specified measure is *motility grade*, where the motility of sperm are divided into four different grades**

- **Grade a**: Sperm with progressive motility. These are the strongest and

swim fast in a straight line

- **Grade b**: (non-linear motility): These also move forward but tend to travel in a curved or crooked motion.

Grade c: These have non-progressive motility because they do not move forward despite the fact that they move their tails.

- **Grade d**: These are immotile and fail to move at all.