III B.Sc Microbiology Honours -III Semester

Course-12 A (IMMUNOLOGY AND MEDICAL MICROBIOLOGY)

Question Bank-2024-25

Essay type questions (Select any Two from each Unit for Internal Choice)

Unit	Q.No	Questions	Marks	BL	CO	PO
Unit 1	1	Define innate and acquired immunity. explain briefly about types of immunity.	8	1 &2	1	
	2	Write about primary organs of immune system	8	2	1	
	3	Write about secondary organs of immune system	8	2	1	
	4	Generalise structure and functions of different cells of immune system	8	3	1	
Unit-2	1	What is meant by antigen and Hapten? Explain about characteristic features of antigens	8	1 &2	2	
	2	Explain the structure and types of antibodies in detail.	8	2	2	
	3	Generalise immune complex formation in vivo by Precipitation, agglutination and neutralization reactions.	8	3	2	
	4	Define and list the outlines of Hypersensitivity reactions.	8	1	2	

Unit	Q.No	Questions	Marks	BL	CO	PO

Unit-3	1	Review about the normal flora present on the human body	8	6	3	
	2	Generalise Opportunistic and Nosocomial infections	4+4	3	3	
	3	Give a general account on causal organism, pathogenesis , diagnosis and prevention of Tuberculosis	8	2	3	
	4	Write about diagnosis and prevention of Hepatitis A and AIDS	4+4	2	3	
Unit-4	1.	Explain the process of collection and handling and processing of clinical samples	8	2	4	
	2	Describe different methods of identification by culturing of clinical samples	8	2	4	
	3	Demonstrate process of PCR and DNA probes in identification of pathogen	8	3	4	
	4	Illustrate role of serological tests in identification of pathogen	8	2	4	

Unit	Q.No	Questions	Marks	BL	CO	PO
Unit -5	1.	Generalise types of vaccines	8	3	5	
	2	Explain the mode of action of Pencilline and streptomycine	8	2	5	
	3	Define antibiotic resistance and explain different methods of microbial antibiotic resistance	8	3	5	
	4	Describe the different tests to evaluate antibiotic susceptibility				

Unit-I :

1. B-cells and T-cells cells involved in acquired immunity (true/False)
2. Which form of immunity conveys the longer-lasting immunity against a infectious agent?
3. Example for natural passive immunity_____
4. What is haemopoiesis?
5. Give two examples for primary organs of lymphatic system----------

UNIT-II

1. Differentiate between epitope and paratope
2. How are the light and heavy chains bound together in antibody?
3. What is the role of MHC?
4. What is MAC in the complement system?
5. What is the nature of a memory cell?

Unit-III

1. Where do the Streptococcus mutans adhere to the body site?
2. Staphylococci are common resident flora of _____.
3. Define Virulence—----------
4. What is the casative agent of Candiosis—---------
5. What is the causative agent of Batulism—------

Unit-IV

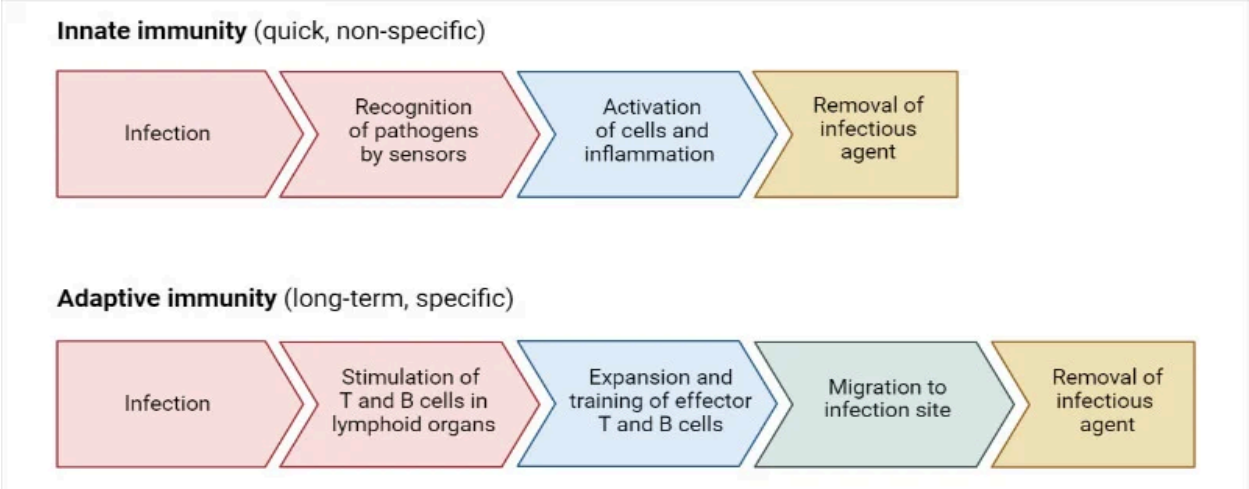
1. Expand IMViC—----------
2. Give example of Selective Medium—---------
3. What is RT-PCR—-------
4. What is WIDAL test—---------
5. What is ELISA_____

Unit-V

1. Who discovered the technique of preparing vaccines from attenuated pathogens and in which year?
2. Give example of Antibiotic Function as Protein Synthesis Inhibitors—---
3. Interferons induce enzyme synthesis in the target cell (True / False)
4. What is antibiotic resistance?
5. What is MIC ?

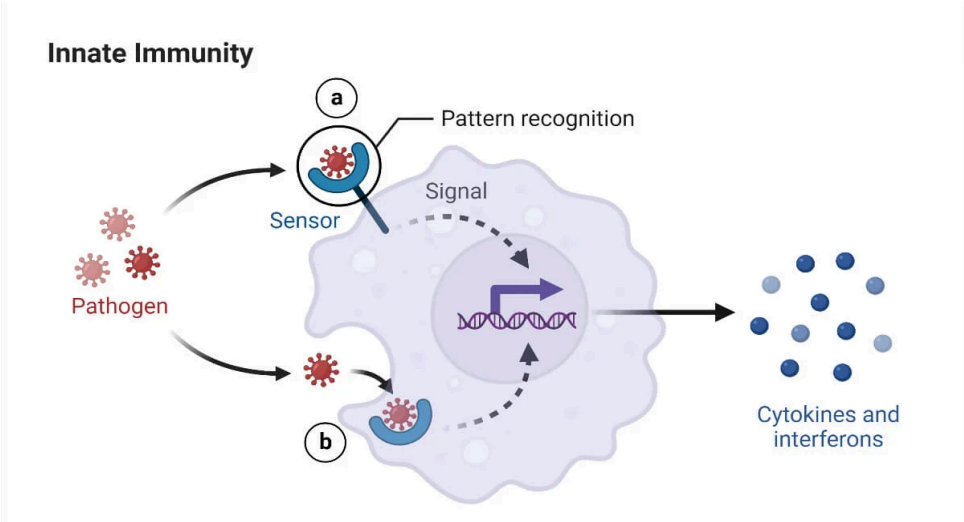
1. **Define innate and acquired immunity. Explain briefly about types of immunity.**

The immune system is the complex network of organs, cells, and proteins that protects the body against infections. This defense system proceeds via the recognition and processing of foreign substances. The network consists of the lymphatic system, complement system, spleen, thymus, bone marrow, and cells (WBCs, B cells, T cells).



INNATE IMMUNITY

The nonspecific arm of the immune system with which an individual is born is termed innate immunity (also called natural immunity). The innate immunity acts as a barrier against foreign invading materials. These barriers form the first line of defense and aid in the activation and modulation of the adaptive immune response.



COMPONENTS OF INNATE IMMUNITY

- **Physiological barriers:** These components include the epithelial lining of the skin, mucous membrane, and physical parameters like temperature, pH, and barriers like enzymes, antimicrobial peptides, cytokines, etc.
- **Anatomical barriers:**
- **Phagocytosis:** It is an endocytosis process in which the foreign body is ingested by specialized cells called phagocytes. Phagosomes are formed when these foreign bodies are ingested, which is a vacuole surrounding these bodies. Later on, the lysosome present within the phagocytes fuses with the phagosome, forming a phagolysosome. The release of lytic enzymes present in lysosome eventually lyses the infectious agent hence clearing the infection.
- **Inflammatory Reactions:** Inflammatory reactions are induced with four cardinal features when host tissue gets damage Like temperature rise,pain,redness and tumor. Histamines, proinflammatory cytokines, defensins, and kinins are mediators of inflammatory reactions.

Types of innate immunity: Includes individual, racial, and species immunity.

- **Individual immunity**: Some individuals of the same race and same species can have varied experiences with certain infections. For example, children are more susceptible to viral fever than adults.
- **Racial immunity**: Individuals of different races within the same species have varied susceptibility or resistance toward infection caused by the same etiological agent. For example, races with sickle cell anemia prevalent in Africans on the Mediterranean coast are resistant to malaria caused by Plasmodium falciparum. This is because sickle cell anemia causes an alteration of the shape of the erythrocyte, which prevents its parasitization.
- **Species immunity**: Individuals from different species have different susceptibilities toward any infection. For example, humans are not affected by chicken cholera, infectious horse anemia, etc., while animals are resistant to many human diseases like syphilis, gonorrhea, measles, etc.

Innate immunity in an individual is also influenced by other factors, such as:

Age: Very old people and young ones are more susceptible to infections when compared to adults.

Hormonal level: Any individual under corticosteroid hormone treatment is more susceptible to infection. Likewise, hormonal disorders like hypothyroidism, diabetes mellitus, etc., can make the individual more prone to infections.

Nutritional status: Nutritional status of the host, like deficiency of vitamins and proteins, makes an individual more susceptible to infections.

Significance of Innate immunity:

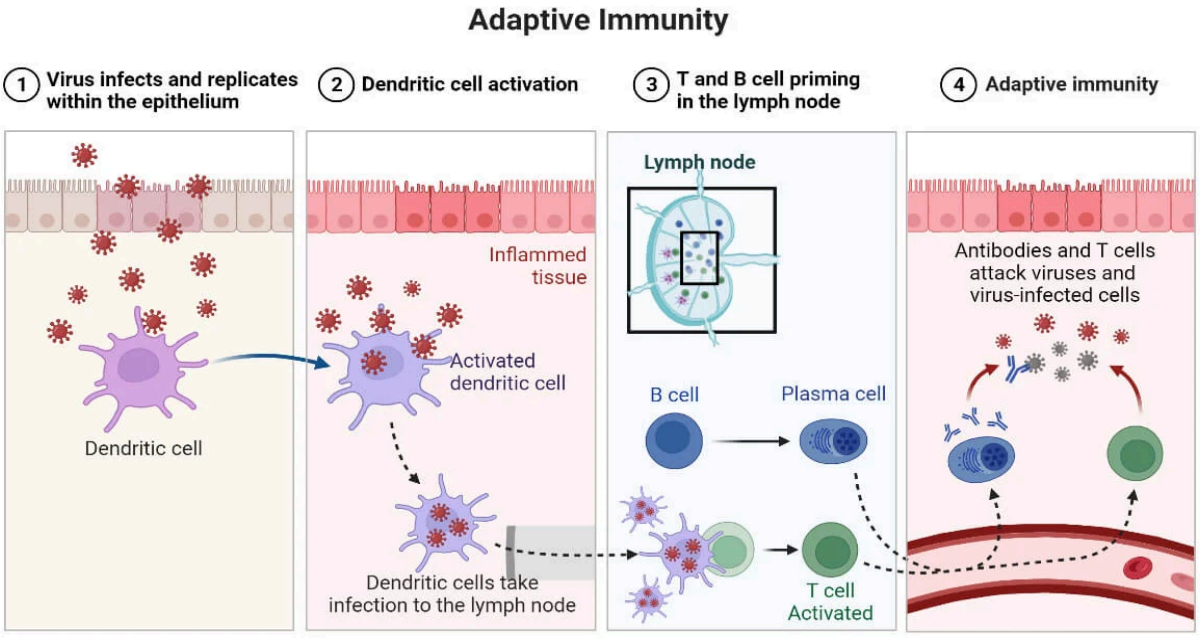
1. Physical and chemical barriers prevent the entry of foreign materials.
2. If the infection is established, a cascade of complement reactions and phagocytosis helps clearance of the infecting agents.
3. It activates the adaptive immune system by the release of cytokines and antigen presentation.

Adaptive or Acquired immunity:

Immunity acquired by an individual during his lifetime is termed acquired immunity. The mediators of acquired infection include:

- **Humoral immunity:** Serum proteins called antibodies produced by B cells mediate this type of immunity. The humoral immune response protects the body against extracellular infections.
- **Cellular immunity:** The immune response in cellular immunity is mediated by T cells. Cellular immunity protects the body against intracellular infections.

In case of a repeated encounter with the same antigen, memory T cells made during initial exposure act by a faster and more potent response.



Types of Acquired immunity:

When a pathogen enters the body, acquired immunity can be induced by the host body or by the artificial transfer of antibodies/ lymphocytes inside the host body.

- **Active immunity:** This is the type of acquired immunity developed in the host body itself because of exposure to the pathogen. When the antigen is recognized by the host cell, an immune response develops, forming antibodies/ helper T cells or cytotoxic T cells.

  Active immunity, when developed by natural infections, is called natural active immunity, whereas when developed upon exposure to preformed vaccines is called artificial active immunity.

- **Passive immunity:** When the host body acquires immunity in the form of preformed serum or lymphocytes, it is called passive immunity. Natural passive immunity is acquired when a mother passes IgG to the fetus during pregnancy, and artificial passive immunity is acquired by the administration of preformed antibodies that help in the neutralization of the antigen.

Significance of Acquired immunity:

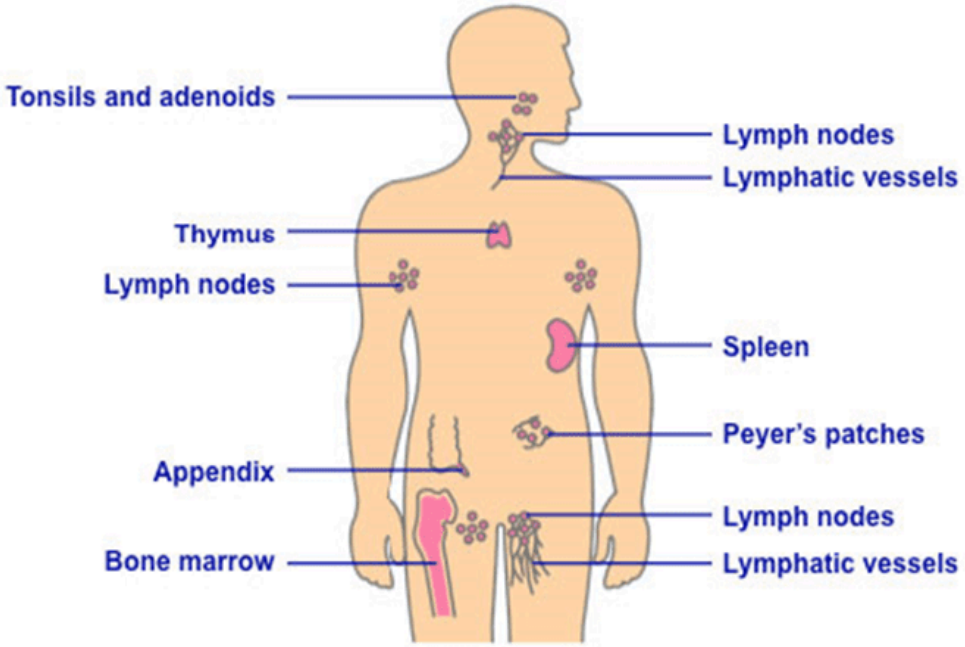
1. When foreign antigens are presented by professional antigen-presenting cells (APCs) or upon release of cytokines from cells, adaptive immunity comes into the act.
2. Antigen-specific antibodies help to neutralize the infection.
3. Immunological memory is developed in the form of memory B cells which respond immediately upon the re-exposure to the specific infectious agent.
4. T helper cells help in the activation of other immune cells.
5. Cytotoxic T cells destroy cells infected with viruses and tumor cells.
6. Regulatory T cells suppress immune response when required, preventing body cells from unwanted self-attack.

2. Write about primary organs of immune system

The lymphatic system is a part of the circulatory system as well as a part of the immune system. It collects the excess body fluid and returns it to the venous circulation so; it is a part of the circulatory system. Similarly, it circulates lymphocytes and plays a key role in lymphocytes-mediated (adaptive) immunity; therefore, it is a part of the immune system. Anatomically, the lymphatic system is made up of two structures; the lymphoid tissues/organs, and the lymphatic vessels.

Lymphatic organs and Tissues:

It comprises organs and specialized tissues that produce and maintains lymphocytes and/or collect lymph and connect it to the bloodstream. The lymphoid organ system can be categorized into primary and secondary lymphoid organs.

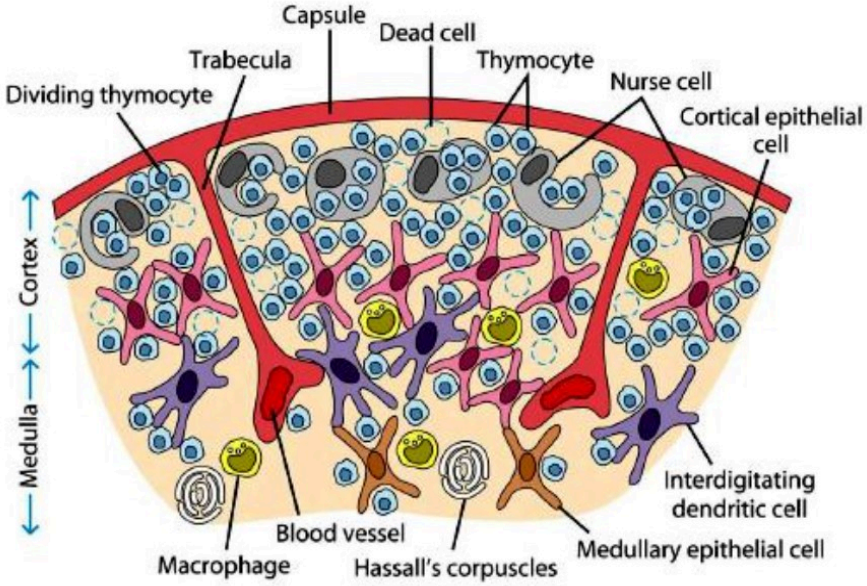


a. Primary Lymphoid Organs

It includes lymphoid organs where the lymphocytes are produced, maintained, and matured. It includes:

Thymus

The thymus is a primary lymphoid organ where T-lymphocytes are matured and undergo the process of positive and negative selection. It is located in the throat in front of the heart, just behind the sternum in the anterior portion of the mediastinum.



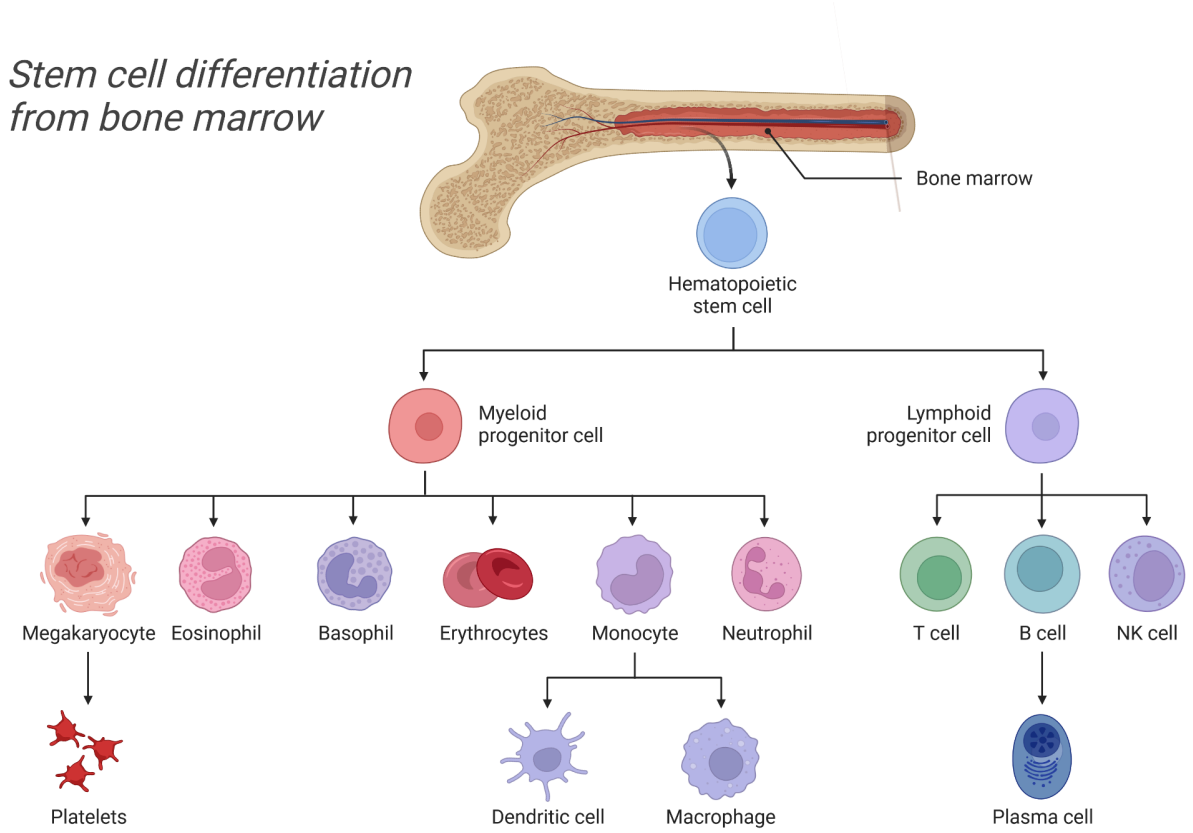
The thymus is functional and distinctly visible up to the early teen (pre-adolescent) age and gradually degenerates and is finally replaced by fat. Hence, it is not found in an adult humans.

Structurally, it is a bilobed organ of about 4 to 6 cm by 2.5 to 5 cm by 1 cm in dimension having a mass of about 50 grams. Each lobe is made of two regions; the outer cortex and the inner medulla. The cortex is made of immature T cells called thymocytes supported by epithelial cells, blood vessels, and other leukocytes. The medulla is mainly made of epithelial cells in form of Hassall's corpuscles.

In the cortex, thymocytes mature into T cells and undergo a selection process to eliminate the T-cells that can target the body's own components. The selected T cells are passed to the medulla where they undergo further maturation and differentiation.

Bone Marrow

Bone marrow is a special semi-solid tissue mass composed primarily of hematopoietic stem cells, adipose tissue, and stromal cells that is present inside the bone in the cancellous section. The red bone marrow is a primary organ of a lymphoid system whose main function is to produce B lymphocytes.



Fetal Liver

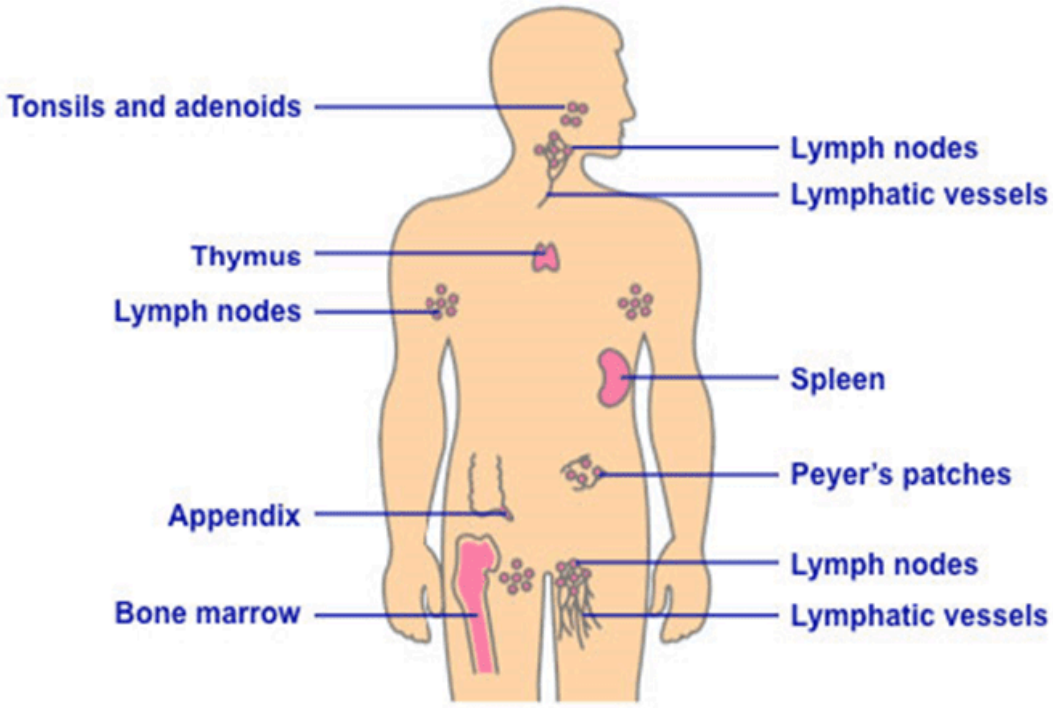
In the fetus stage, the liver cells produce lymphocytes; hence, the fetus's liver is a primary organ of the fetal lymphatic system. However, the liver of infants and adults does not produce any lymphocytes.

Bursa of Fabricius:

The bursa of Fabricius is a unique, primary lymphoid organ found in birds. It is a specialized structure connected to the cloaca, the common chamber for the intestinal, urinary, and genital tracts. It's essential for B cell development and antibody production, playing a crucial role in the bird's immune system.

3. Write about secondary organs of immune system

The lymphatic system is a part of the circulatory system as well as a part of the immune system. It collects the excess body fluid and returns it to the venous circulation so; it is a part of the circulatory system. Similarly, it circulates lymphocytes and plays a key role in lymphocytes-mediated (adaptive) immunity; therefore, it is a part of the immune system. Anatomically, the lymphatic system is made up of two structures; the lymphoid tissues/organs, and the lymphatic vessels.



Lymphatic organs and Tissues:

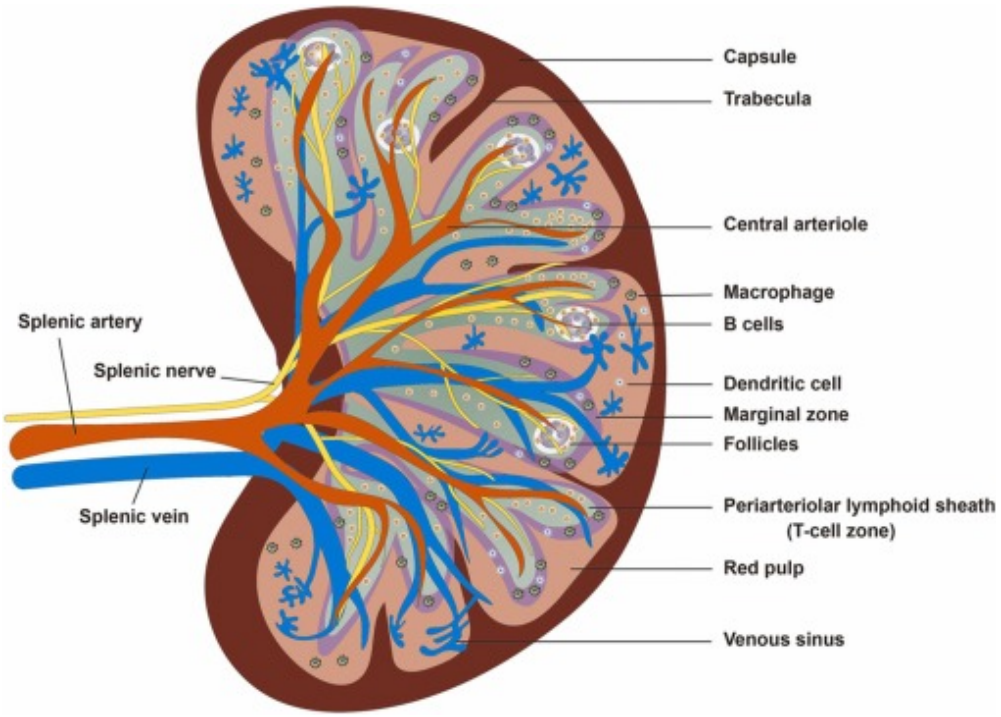
It comprises organs and specialized tissues that produce and maintains lymphocytes and/or collect lymph and connect it to the bloodstream. The lymphoid organ system can be categorized into primary and secondary lymphoid organs.

Secondary Lymphatic organs:

It includes lymphoid organs where the lymphocytes undergo further maturation and contact the bloodstream with the lymph. It includes:

Spleen

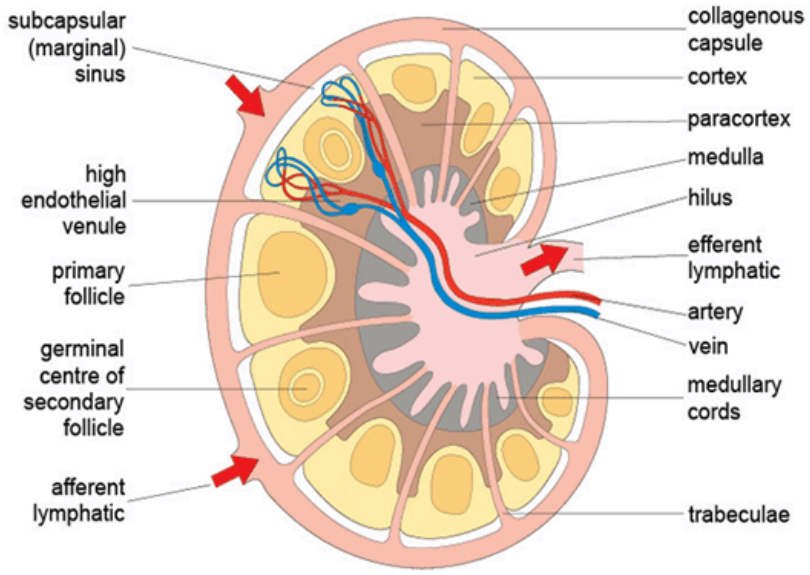
The spleen is the blood filter of vertebrates found at the back of the stomach in the abdominal cavity below the diaphragm. It is a large organ or about 13 cm by 8 cm by 3 cm in dimension and weighs about 200 grams.. It contains two distinct types of tissues; the red pulp and the white pulp. The red pulp plays the primary function of filtering the blood, removing the old RBCs, and digesting the hemoglobin. The white pulp primarily contains lymphocytes (both B and T lymphocytes). It is a site where lymphocytes are activated by the antigens present in the blood resulting in the activation of the humoral and cell-mediated immune response.



Lymph Nodes

Lymph nodes are encapsulated small kidney-shaped mass of lymphatic tissues distributed throughout the body along the network of lymphatic vessels which filters the lymph and stores lymphocytes. They are numerous (more than 600 in an adult human) small nodes of varied sizes ranging from 2 to 25 mm in length. They are prevalent in the armpits (Called the axillary lymph nodes), groins (called the inguinal lymph nodes), neck (called the cervical lymph nodes), and knees (called the popliteal lymph nodes).

The lymph node is divided into two regions; the inner medulla and the outer cortex externally covered by a fibrous capsule. Each lymph node is connected with multiple afferent lymphatic vessels in its convex side from where lymph enters the node. On the concave side, there is an efferent lymphatic vessel from where filtered lymph leaves the node.



The lymph nodes filter the lymph and identify antigens or pathogens present in the circulation and activate the cell-mediated immune responses if any foreign bodies are detected. They harbor lymphocytes and regularly pass them into blood circulation for immune surveillance and response.

Mucosa-associated Lymphatic Tissues

These include diverse types of lymphoid tissue masses present in the mucosal surface of several organs. They are primarily located in organs and mucosal surfaces of the gastrointestinal, respiratory, and genitourinary systems. It Contains lymphocytes (T cells, B cells), plasma cells, macrophages, and specialized M cells in the gut.

Function: Plays a vital role in initiating immune responses to antigens encountered at mucosal surfaces, including the production of IgA antibodies. **Examples:**Includes

tonsils, Peyer's patches in the small intestine, and the appendix. **Importance:**Constitutes a significant portion of the body's lymphoid tissue and is essential for maintaining mucosal immunity. Includes gut-associated lymphoid tissue (GALT), bronchial/tracheal-associated lymphoid tissue (BALT), and nose-associated lymphoid tissue (NALT).

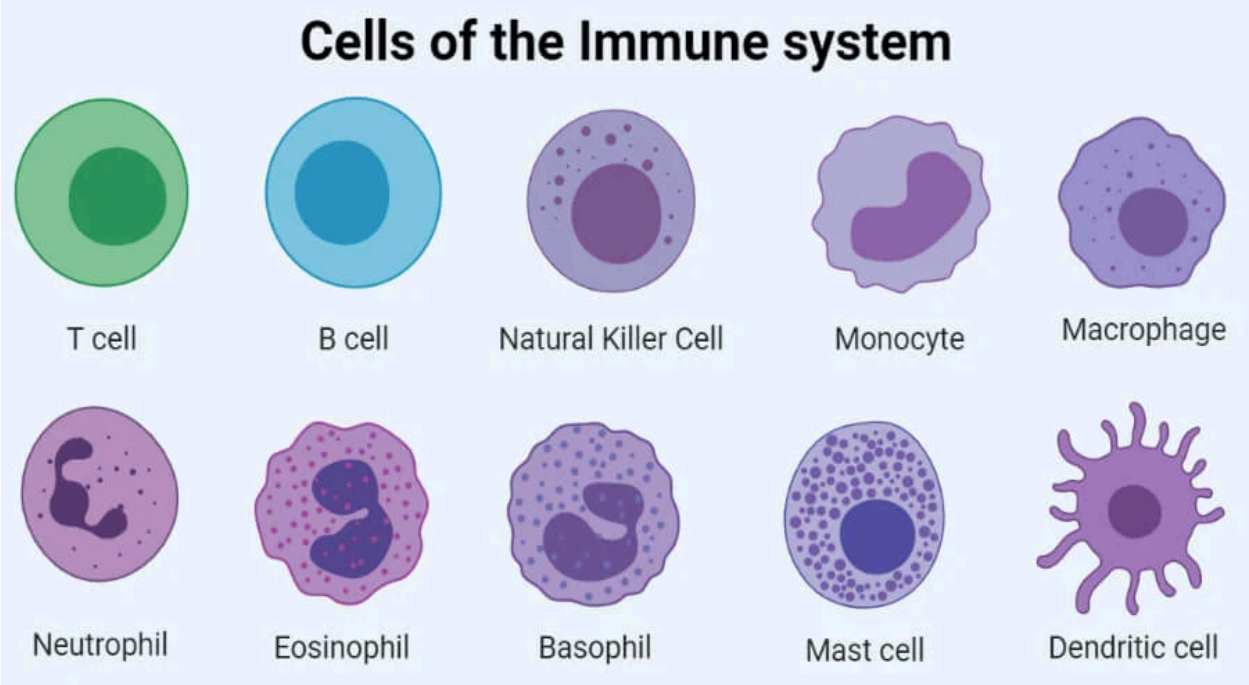
Lymph Vessels:

Lymphatic vessels also called lymph vessels or lymphatics, are the closed thin-walled tubes that form a network of ducts interconnecting the lymphoid organs through which lymph circulates. Lymph vessels are one-way vessels that collect and transport lymph from body tissues to the heart. There are several lymphatic mini-valves that check the backflow of lymph and maintain the unidirectional flow of lymph.

4. Generalise structure and functions of different cells of immune system

Cells of Immune system

The lymphocytes are the central cells of the immune system which are responsible for adaptive immunity and immunological response, specificity, memory, and self/non-self recognition. They also function to engulf and destroy micro-organisms, present antigens and secrete cytokines.



1. B-lymphocytes

- They are the specialized cells of the immune system whose major function is to produce antibodies also known as immunoglobulins or gamma globulins.

- B-lymphocytes are synthesized and mature in the bone marrow from the hematopoietic stem cells, and after which they mature, migrate, and express themselves by forming unique antigen-binding receptors on their membranes, known as B-cell receptors or antibodies.
- Migration of mature B-cells moves to the bone marrow, lymph nodes, spleen, some parts of the intestines, and the bloodstream.

- When B-cell interacts with an antigen for the first time and it has to match membrane-bound receptors (antibodies), the antibodies bound to the B-cell bind the antigen causing the B-cell to divide rapidly, and its progenitors to differentiate into **memory B-cells and effector B-cells known as plasma cells.**
- The plasma cells are responsible for producing the antibodies that can be secreted into the bloodstream, tissues, respiratory secretions, intestinal secretions, and tears.
- The plasma cells have a short life span of a few days but they secrete large amounts of antibodies during this time, with approximately 2000 molecules of antibodies per plasma cell per second.
- The antibody molecules are specifically designed for every foreign antigen they encounter and interact like a lock and key mechanism.

2. T-Lymphocytes

- T-lymphocytes are also known as T-cells, often named in lab reports as CD3 cells
- They also arise in the bone marrow but migrate to the thymus gland for maturation, where they express a unique antigen-binding molecule on its membrane known as the T-cell receptor.
- The name **T** originated from its site of maturation, the **Thymus.**
- Mature T-cells leave the thymus and populate other organs of the immune system, such as the spleen, lymph nodes, bone marrow, and blood.
- Unlike the B-cell receptors that can recognize antigens alone, T-cell receptors only recognize antigens that are bound to cell membrane proteins known as Major Histocompatibility Complex (MHC) molecules.

- The T-cells are classified into three categories: **T helper (Th), T cytotoxic (Tc), and T suppressor (Ts) cells.**
- The Th and Tc cells are differentiated from each other with the **presence of their CD4 and CD8 membrane glycoproteins** on their surfaces.
- T cells naturally **displaying CD4 function as T helper (Th) cells** while those **displaying CD8 naturally function as T cytotoxic (Tc)** cells.
- The Th cells recognize and interact with antigens that are presented on the MHC class II molecule complex, then they become activated becoming effector cells that are able to secrete various growth factors that are collectively known as **cytokines.**
- The cytokines that are secreted are actively involved in the activation of B-cells, T-cytotoxic cells, macrophages, and other immune cells.

- The cytokine patterns produced by the activated TH -cells result in different immune responses. The Th -derived cytokines enable the recognition of an antigen-MHC class I molecule complex by the Tc cells which then proliferate and differentiate into effector cells known as **Cytotoxic T-lymphocytes (CTL).**

3. Natural killer cells (NK cells)

- These are large granular lymphocytes, that do not express surface markers like the B and T-cell lineages
- These cells also indicated that they play key roles in host defense against tumor cells and cells infected with some, not all viruses.
- They constitute 5-10% of lymphocytes in the human peripheral Their ability to recognize antigens is based on two mechanisms:
- They can employ NK cell receptors to distinguish abnormalities such as a reduction in the expression of class I MHC molecules and the abnormal profile of the surface antigens that are displayed by some tumor cells and cells infected by some viruses.
- Secondly, the NK cells also recognize potential target cells which are tumor cells and cells that are infected by viruses. These target cells display antigens against which the immune system has already produced antibody response to as antitumor or antiviral antibodies, that bind to the surfaces of these targets.
- The NK cells express membrane receptors called CD16, which are receptors for the carboxyl-terminal end of the IgG molecule, Fc region. The NK CD16 receptors attach to these antibodies and destroy the targeted cells subsequently, by a mechanism known as the Antibody-dependent cell-mediated cytotoxicity (ADCC).

Macrophages:(Mononuclear phagocytes)

- These are immune cells i.e **monocytes** that are freely circulating in blood and **macrophages** that are found in the tissues.
- During hematopoiesis in the bone marrow, **granulocyte-monocyte progenitor cells** differentiate into **promonocytes**, which leave the bone marrow and enter the blood, where they differentiate further into **mature monocytes**.
- Monocytes circulate in the bloodstream for about 8 h, during which they enlarge and then migrate into the tissues and differentiate into specific tissue macrophages or into dendritic cells.
- Differentiation of monocyte into a tissue macrophage involves a number of changes,The cell enlarges five- to tenfold.
- Its intracellular organelles increase in both number and complexity
- It acquires increased phagocytic ability and produces higher levels of hydrolytic enzymes. It begins to secrete a variety of soluble factors.

- Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages.
- Free macrophages travel by amoeboid movement throughout the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:
    - Alveolar macrophages in the lung
    - Histiocytes in connective tissues
    - Kupffer cells in the liver
    - Mesangial cells in the kidney

Some of the functions of macrophages include:

- Phagocytosis -Phagocytosis of bacteria, viruses, and other foreign particles is the most important function of macrophages. The macrophages on their cell surfaces have Fc receptors that interact with the Fc component of the IgG, thereby facilitating the ingestion of the opsonized organisms. They also have receptors for C3b, another important opsonin. After ingestion, the phagosome containing the microbe fuses with a lysosome. The microbe within the phagolysosome is killed by reactive oxygen, reactive nitrogen compounds, and lysosomal enzymes.
- Antimicrobial and cytotoxic activities include the oxygen-dependent and oxygen-independent cytotoxicity/killing.
- Antigen processing – After ingestion and degradation of foreign materials, the fragments of antigen are presented on the macrophage cell surface in conjunction with class II MHC proteins for interaction with the TCR of CD4+ helper T cells.
- Secretion of growth factors important for the development of an immune response such as cytokines, such as interleukin 1 (IL-1), TNF-α, and interleukin 6 (IL-6), that promote inflammatory responses, complement proteins, hydrolytic enzymes, and a cascade of Tumor Necrotic Factors, TNF-α (GM-CSF, G-CSF, M-CSF) that induce and kill tumor cells and promote hematopoiesis.

Granulocytes:

- Granulocytes are white blood cells (leukocytes).
- They are classified based on their cellular morphologies and cytoplasmic staining characteristics and they include **neutrophils, eosinophils, basophils, or mast cells.**
- All granulocytes have multilobed nuclei that make them visually distinctive and easily distinguishable from lymphocytes, whose nuclei are round. The cytoplasm of all granulocytes is replete with granules that are released in response to contact with pathogens.
- These granules contain a variety of proteins with distinct functions: Some damage pathogens directly; some regulate trafficking and activity of other white blood cells,

including lymphocytes; and some contribute to the remodeling of tissues at the site of infection.

- Neutrophils have a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a **polymorphonuclear leukocyte (PMN)** for its multilobed nucleus.
- The eosinophils have a bilobed nucleus and a granulated cytoplasm that stains with the aciddye eosin red (hence its name).

- The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue.
- Both neutrophils and eosinophils are phagocytic, whereas basophils are not.
- Neutrophils constitute the majority (50% to 70%) of circulating leukocytes and are much more numerous than eosinophils (1%–3%), basophils (≤ 1%), or mast cells (≤ 1%).

Neutrophils

- Neutrophils are produced by hematopoiesis n the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days.
- In the bone marrow, a surmountable level of neutrophils is produced in response to the types of infections and they are normally the first cells that arrive at the site of inflammation.
- The resulting transitory increases in the number of circulating neutrophils known as leukocytosis, which is an indicator of an infection, medically.
- Neutrophils exhibit a larger respiratory burst than macrophages and they are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates.
- Neutrophils also express higher levels of defensins than macrophages.

Basophils

- Basophils are nonphagocytic granulocytes containing large granules that are filled with basophilic proteins that stain blue in standard H & E staining methodologies.
- Naturally, basophils are in the body's normal circulation but they can be very potent.
- They function by binding to circulating antibodies and react by the content of their granules which are pharmacologically active substances found in their cytoplasm.
- These substances play a major role in certain allergic responses. For example, histamines are the most common and well-known protein in that basophilic granules. They play a role in increasing blood vessel permeability and smooth muscle activity.

Mast cells:

- Mast cells are formed in the bone marrow.

- They are released from the bone marrow into the blood as undifferentiated cells, and when they enter the tissues they then mature.
- Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances.
- Mast cells also play an important role in the development of allergies.

Dendritic cells:

- The dendritic cells acquire their name because they are covered with long membrane extensions resembling the dendrites of the nerve cells.
- Their membranous extension extends and retracts dynamically, increasing the surface area available for browsing lymphocytes.
- They are very diverse according to research, and they seem to arise from both the myeloid and lymphoid lineages of hematopoietic cells.
- Dendritic cells generally perform the distinct functions of antigen capture in one location and antigen presentation in another.
- Outside lymph nodes, immature dendritic cells monitor the body for signs of invasion by pathogens and capture intruding or foreign antigens.
- They then process these antigens, then migrate to lymph nodes, where they present the antigen to naïve T cells, initiating the adaptive immune response in addition to phagocytosis.

Unit-II

1. What is meant by antigen and Hapten? Explain about characteristic features of antigens

Definitions:-

Antigen

Antigen is defined as any substance which when introduced in the body, stimulates the production of an antibody with which it reacts specifically. Its ability to bind with antibodies or T-cell is referred to as antigenicity.

Immunogen is substance which produces an immune response as well as binds to its products i.e., antibodies or sensitized T-cells, when injected into the host.

Hapten refers to a group of substances, usually very small in size, which do not induce an immunresponse by themselves alone. But if combined with another molecules called carries, the hapten-carrier complex induces an immune response

Types:

Exogeneous Antigen – These enters the body from outside i.e external environment. Common examples includes microorganisms, drugs, pollen, pollutants or even food items etc.

Endogenous Antigens - These antigens are produced within the host.

Based on genetic consideration antigens are divided into three types:

Autoantigens, alloantigens and heteroantigens
Autoantigens These are the antigens belonging to the host itself.

Alloantigens These are the antigens derived from other members of species of the host, but not from the host itself. Such antigens are important in tissue transplant and blood transfusion processes e.g, antigens present on donor and the recipient RBCs are alloantigens to each other.
Heteroantigens These antigens are from two different species such as plants and animals or microorganisms etc.
The smallest unit of antigenicity is known as the antigenic determinant or epitope. The epitope is that small area on the antigen usually consisting of four or five aminoacid or monosaccharide residues, possessing a specific chemical structure, electrical charge and steric configuration, capable of sensitising an immunocyte and of reacting with its complementary site on the specific antibody or T cell receptor. The combining area on the antibody molecules, corresponding to the epitope, is called the paratope.

DETERMINANTS OF ANTIGENICITY
A number of properties that make a substance antigenic have been identified but the exact basis of antigenicity is still not clear
Size : Antigenicity is related to the molecular size. Very large molecule are highly antigenic and particles with low antigenicity are nonantigenic. Low molecular weight substances may be rendered antigenic by adsorbing them on a large inert particles such as bentonite or kaolin.

Chemical nature : Proteins and polysaccharides are good immunogen as compared to lipids and nucleic acids, Among them proteins are better than carbohydrates. Nucleic acids, poor by themselves, can generate response in combination with other substances.

Susceptibility to tissue enzymes : Only substances which are metabolized and are susceptible to the action of tissue enzymes behave as antigens. Antigens introduced into the body are degraded by the host into fragments of appropriate size containing the antigenic determinants.

Foreignness : Only antigen which are ‘foreign’ to the individual (nonself) induce an immune response. The antigenicity of a substance is related to the degree of its foreignness. Antigen from related species are less antigenic than those from distant species.

Antigenicity specificity : The basis of antigenic specificity is a stereochemical .Crossreaction can occur between antigen that bear stereochemical similarities. In some instances, apparent cross reactions may actually be due to the sharing of identical antigenic determinants by different antigens.

Species specificity Tissues of all individuals in a species contain species- specific antigens. There exhibits some degree of cross - reaction between antigens of related species.

Isospecificity Isoantigens are antigens found in some but not all members of a species. The species may be grouped depending on the presence of different isoantigens in its members

Autospecificity Autologous or self antigens are ordinarily nonantigenic but there are exceptions. Sequestrated antigens that are not normally found free in circulation or tissue fluids are not recognised as a self antigens.. Similarly, antigens that are absent during embryonic life and develop later are also not recognized as self antigens.
Some organs, such as the brain, kidney and lens protein of different species, share the same antigen. Such antigens, characteristic of organ or tissue and found in different species, are organ – specific antigens.

Heterogenetic (heterophile) specificity The same or closely related antigens may sometimes occur in different biological species, classes and kingdoms. These are known as heterophile antigens .

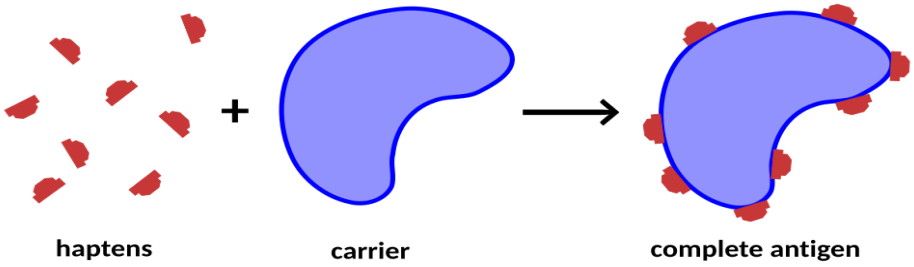
Super-Antigens
When the immune system encounters a conventional T-dependent antigen, only a small fraction (1 in 104 -105) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response). However, there are some antigens which polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called superantigens. This response leads to overproduction of Th cytokines which lead to systemic toxicity. Superantigens can be viral proteins (called endogenous superantigens) or they can be bacterial superantigens (called the exogenous superantigens). Examples: Staphylococcal enterotoxins (causes food poisoning), Streptococcal pyrogenic exotoxins (causes shock).

Haptens:

Haptens Derived from Greek word “Haptein” which means “ To fasten”. The term Hapten was first coined by Karl Landsteiner. Many low molecular weight organic molecules that are not antigenic by themselves but become antigenic if they bond to a layer carrier molecule such as a protein. These low molecular weight compounds require carrier molecules to act as a complete Antigen.The carrier molecule is a non antigenic component and helps in provoking the immune response.

Example: Serum protein such as Albumin or Globulin. Low molecular weight (less than 10,000).

Haptens can react specifically with its corresponding antibody. Exampless: Capsular polysaccharide of pneumococcus , Polysaccharide “C” of beta helaemolytuc streptococci, cardiolipin antigen , etc.



- Haptens may be complex or simple.
- Complex Hapten : Polyvalent and Precipitate with specific antibodies.
- Simple Haptens : Univalent and . Non – Precipitate with specific antibodies.
- Examples for Haptens
- 1. Aniline (an organic compound) and it’s derivatives (o-, m, p- benzoic acid)
- 2. Hydralazine - A blood pressure lowering drug.
- 3. Fluorescein – A fluorescent dye.
- 4. Penicillin – An antibiotic.

2. Explain the structure and types of antibodies in detail.

Antibodies

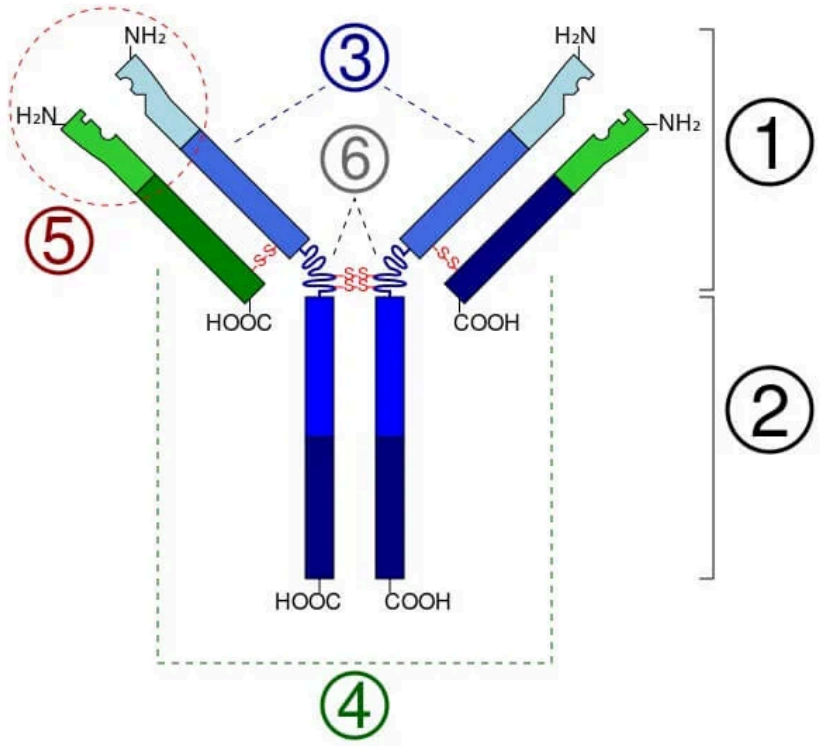
Antibodies are protein molecules naturally produced or synthesized by the B-lymphocytes.

They are also known as Immunoglobulins. The use of the term antibody defines an Immunoglobulin molecule that has specificity for an epitope of the molecules that make up antigens.

Produced and secreted by plasma cells, antibodies are soluble molecules that travel throughout the body to find and bind to their targets which are foreign substances known as antigens.

- Antibodies have specific sites that recognize antigen epitopes known as antigen-binding sites. These are the regions on the antibodies where the antigen binds on the antibody, and antigens have special surface structures known as epitopes or antigen determinants which are arranged discontinuously and they vary or are different.

This interaction involves a number of weak forces including the hydrogen bonds, hydrophobic interactions, electrostatic forces, and the van der Waals forces.



1- Fab region. **2-** Fc region. **3-** Heavy chain (blue) with one variable (VH) domain followed by a constant domain (CH1), a hinge region, and two more constants (CH2 and CH3) domains.
4- Light chain (green) with one variable (VL) and one constant (CL) domain.
5- Antigen binding site (paratope).
6- Hinge regions

Structure of Antibody Molecule

Understanding the structure of antibody molecules is crucial to appreciate antibody structure and function. Each antibody typically has a Y-shaped configuration made of four polypeptide chains:

1. **Two Heavy (H) Chains**: These are longer polypeptide chains that determine the class (isotype) of the immunoglobulin.
2. **Two Light (L) Chains**: These are shorter and help form the antigen-binding sites.

Immunoglobulin Structure

When we talk about immunoglobulin structure, the chains are held together by disulphide bonds and other non-covalent interactions. The main regions of an antibody molecule are:

- **Variable (V) Region**: Present at the tips of the Y shape, forming the antigen-binding sites. This region differs from one antibody to another, enabling specificity for unique antigens.

- **Constant (C) Region**: The stem of the Y shape and portions of the arms that do not vary significantly among antibodies of the same class. This region determines how the antibody interacts with other components of the **immune system**.

Key Functional Segments

- **Fab (Fragment Antigen-Binding)** Region: The two 'arms' of the Y that bind specifically to the antigen.
- **Fc (Fragment Crystallisation)** Region: The 'stem' that interacts with various immune cells (like macrophages) and complements proteins, thus triggering a wider immune response.

By studying the structure of antibodies, we learn how the molecule locks onto an antigen and instigates its elimination.

Types of Antibodies and Their Functions

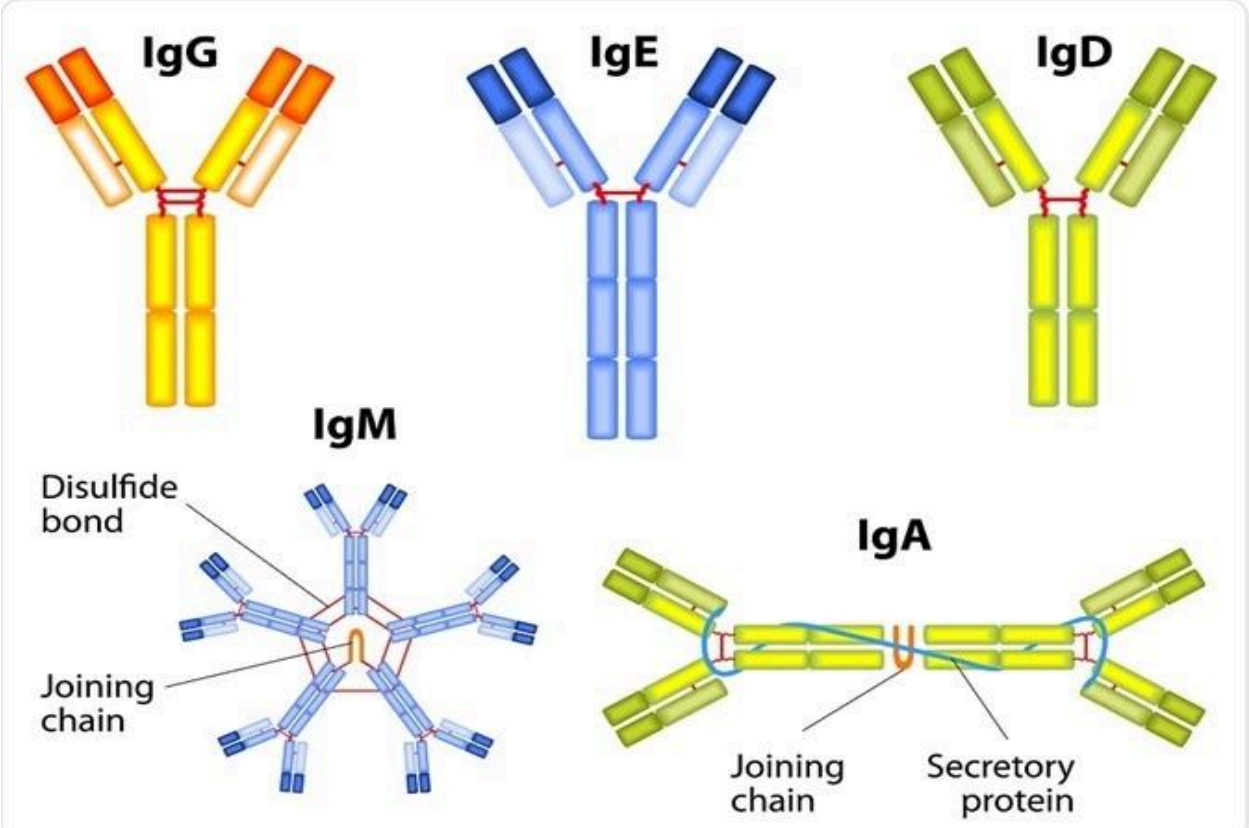
One of the most important aspects of immunoglobulin structure is that it allows for different classes (isotypes) of antibodies. Each class has distinct roles and unique properties. Let's explore the types of antibodies and their functions:

1. IgM

- **First Responder**: IgM is produced first upon initial exposure to an antigen.
- **Pentameric Form**: Usually circulates as five units joined together, making it effective at clumping pathogens.
- **Functions**: Facilitates agglutination (clumping of antigens) and activates the complement system, thus enhancing pathogen destruction.

2. IgG

- **Most Abundant**: Constitutes about 80% of the total antibody content in the blood.
- **Crosses Placenta**: IgG is the only antibody that can cross the placenta, providing foetal immunity.
- **Functions**: Neutralises toxins, supports phagocytosis, and offers long-term protection



3. IgA

- **Secretory Antibody**: Predominantly found in saliva, tears, breast milk, and intestinal fluids.
- **Dimeric in Secretions**: Often present as two units linked together, especially in bodily secretions.
- **Functions**: Acts as the first line of defence on mucosal surfaces, preventing the attachment of pathogens to epithelial cells.

4. IgD

- **Receptor on B Cells**: Found mainly bound to the surface of B lymphocytes.
- **Functions**: Plays a key role in the activation and regulation of B cells.

5. IgE

- **Least Abundant**: Accounts for a very small fraction of antibodies in circulation.
- **Allergic Reactions**: Responsible for immediate hypersensitivity reactions (e.g., pollen allergies).
- **Functions**: Binds to allergens and triggers histamine release from mast cells, causing inflammation.

When learning about the types of antibodies and their functions, remember that each class is essential in different defence strategies, ensuring a comprehensive immune response.

3. Generalise immune complex formation in vivo by Precipitation, agglutination and neutralization reactions.

Immune complexes form when antibodies bind to antigens. This process is a crucial part of the body's defense against pathogens and other foreign substances. While typically a beneficial part of the immune response, their formation and subsequent deposition in tissues can sometimes lead to inflammation and tissue damage, particularly when the antigen-antibody ratio is imbalanced or when they deposit in specific locations.

Properties of Antigen and antibody interactions:

- Highly specific reaction
- Occurs in an observable manner
- Non-covalent interaction (Van der Waals forces, Ionic bonds, Hydrogen bonds, Hydrophobic interactions)
- No denaturation of antibodies and antigens
- Reversible

Affinity: It is the strength with which one antigen binds on a single antigen-binding site on an antibody.

Avidity: It is a broader term than affinity. It is a measure of the overall strength of the Ag-Ab complex. It depends on:

- The affinity of the antibody
- Valency(no. of binding sites) of antibody and antigen
- And the structural arrangement of epitopes and paratopes.

Cross-Reactivity: It refers to the ability of an antibody to bind to similar epitopes of different antigens.

Ag-Ab reactions are basically of two types:

1. In Vivo (Occurring in natural condition): It includes the general antibody-mediated immune response occurring in our body against any antigen.

- Agglutination
- Precipitation
- Complement fixation
- Neutralization
- Ab Dependent Cell-Mediated Toxicity
- Phagocytosis/Opsonisation

2. In Vitro (Done in artificial conditions): It includes a series of serological tests performed in laboratories to detect antigens or antibodies in case of many diseases.

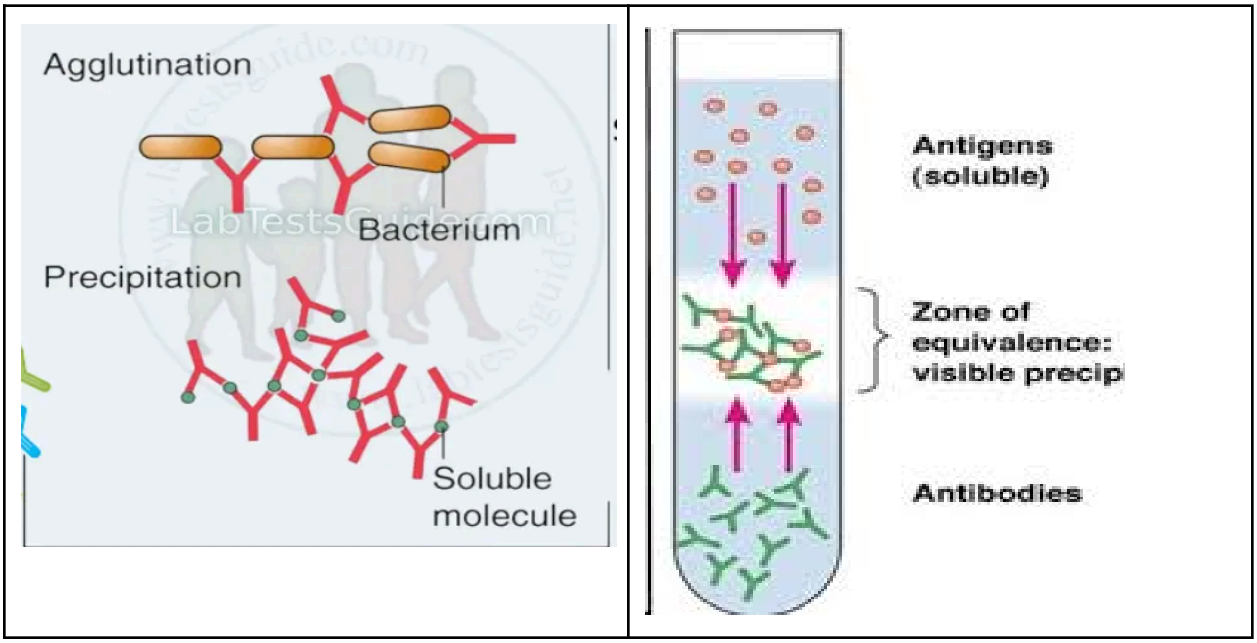
Precipitation reactions:
Precipitation Reaction is a type of antigen-antibody reaction, in which the antigen occurs in a soluble form. When a soluble antigen reacts with its specific antibody, at an optimum temperature and PH in the presence of electrolyte antigen-antibody complex forms insoluble precipitate.
Antigen (soluble) + Antibody (soluble) → Ag-Ab complex (insoluble)

The proportion of Ag and Ab in the reaction must be equivalent for the precipitation reaction to occur. The zone at which there are equivalent numbers of Ag and Ab in solution is called the **zone of equivalence**.

A precipitation reaction is interfered below or above the zone of equivalence due to obstruction in a lattice formation. The zone below the zone of equivalence i.e. where antigens are lesser than antibodies is called the **prozone phenomenon**.

The zone above the zone of equivalence i.e where antigens are larger in number than antibodies is called the **post zone phenomenon**.

There are several precipitation methods applied in clinical laboratory for the diagnosis of disease. Precipitation methods include double immunodiffusion, radial immunodiffusion and electroimmunodiffusion. The most commonly used serologic precipitation reactions are the Ouchterlony test (based on double immunodiffusion and named after the Swedish physician who invented it), and the Mancini method (based on single radial immunodiffusion).



Agglutination Reactions:

It occurs optimally when antigens and antibodies react in equivalent proportions. This reaction is analogous to the precipitation reaction in that antibodies act as a bridge to form a lattice network of antibodies and the cells that carry the antigen on their surface. Because cells are so much larger than a soluble antigen, the result is more visible when the cells aggregate into clumps. When particulate antigens react with specific antibody, antigen-antibody complex forms visible clumping under optimum PH and temperature. Such a reaction is called agglutination. Antibodies that produce such reactions are called **agglutinins**.

Types of Agglutination:

- **Direct Agglutination:** The antigen is naturally part of the particle (e.g., red blood cells in blood typing).
- **Indirect/Passive Agglutination:** The antigen is artificially attached to a carrier particle (e.g., latex beads).

Examples of agglutination tests:

- **Slide/Tile agglutination:** Basic type of agglutination reaction that is performed on a slide. Identification of bacterial types represents a classic example of a slide agglutination. In this method suspension of unknown antigen is kept on slide and a drop of standardized antiserum is added or vice versa. A positive reaction is indicated by formation of visible clumps. E.g. Widal test, RPR test.
- **Tube agglutination:** It is an agglutination test performed in a tube and standard quantitative technique for determination of antibody titre. In this method serum is diluted in a series of tubes and standard antigen suspensions (specific for the suspected disease) are added to it. After incubation, antigen-antibody reaction indicates visible clumps of agglutination.
- **Antiglobulin (Coombs) test:** This test was devised by Coombs, Mourant, and Race for detection of incomplete anti-Rh antibodies that do not agglutinate Rh+ erythrocytes in saline. When serum containing incomplete anti-Rh antibodies is mixed with Rh+ erythrocytes in saline, incomplete antibody antiglobulin coats the surface of erythrocytes but does not cause any agglutination. When such erythrocytes are treated with antiglobulin or Coombs serum (rabbit antiserum against human gamma globulin), then the cells are agglutinated.

Applications:

- **Blood Typing:** ABO and Rh blood group typing relies on agglutination reactions.
- Identification of Bacteria. E.g. Serotyping of *Vibrio cholera*, Serotyping of *Salmonella* Typhi and Paratyphi.
- Serological diagnosis of various diseases. E.g Rapid plasma regains **(RPR)** test for Syphilis, Antistreptolysin O **(ASO)** test for rheumatic fever.
- Detection of unknown antigen in various clinical specimens. E.g. detection of **Vi** antigen of *Salmonella* Typhi in the urine.

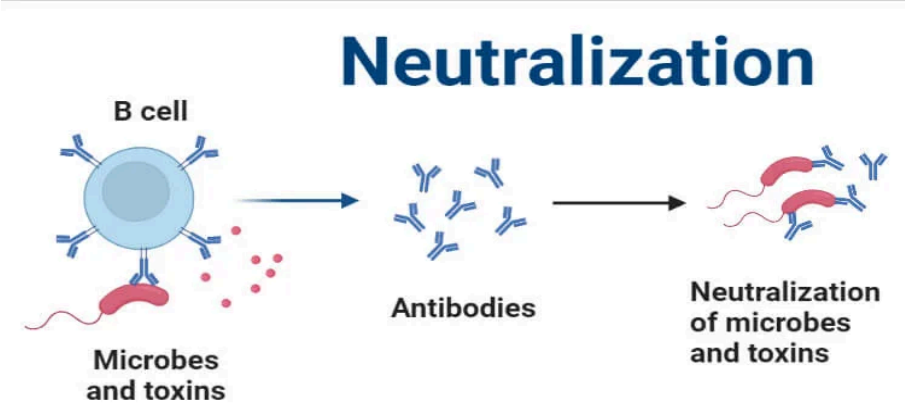
Neutralization :

A neutralizing antibody defends a cell from an antigen or infectious body by inhibiting or neutralizing any effect it has biologically. The antibody response is crucial for preventing many viral infections and may also contribute to the resolution of an infection. When a vertebrate is infected with a virus, antibodies are produced against many epitopes of multiple virus proteins. A subset of these antibodies can block viral infection by a process called neutralization. This usually involves the formation of a virus-antibody complex.

This virus-antibody complex can prevent viral infections in many ways. It may interfere with virion binding to receptors, block uptake into cells, prevent uncoating of the genomes in endosomes, or cause aggregation of virus particles. Many enveloped viruses are lysed when antiviral antibodies and serum complement disrupt membranes. Antibodies can also neutralize viral infectivity by binding to cell surface receptors.

Neutralizing antibodies have shown potential in the treatment of retroviral infections.

In diagnostic immunology and virology laboratories, the evaluation of neutralizing antibodies, which destroy the infectivity of viruses, can be measured by cytopathic effect and haemoagglutination inhibition tests.



4. Define and list the outlines of Hypersensitivity reactions.

HYPERSENSITIVITY REACTIONS AND TYPES

Hypersensitivity (also called hypersensitivity reaction or intolerance) is an abnormal physiological condition in which there is an undesirable and adverse immune response to an antigen.[1][2] It is an abnormality in the immune system that causes immune diseases including allergies and autoimmunity.

there are four types of hypersensitivity, namely: type I, which is an Immunoglobulin E (IgE) mediated immediate reaction; type II, an antibody-mediated reaction mainly involving IgG or IgM; type III, an immune complex-mediated reaction involving IgG, complement system and phagocytes; and type IV, a cytotoxic, cell-mediated, delayed hypersensitivity reaction involving T cells.

The first three types are considered immediate hypersensitivity reactions because they occur within 24 hours. The fourth type is considered a delayed hypersensitivity reaction because it usually occurs more than 12 hours after exposure to the allergen, with a maximal reaction time between 48 and 72 hrs.

Type-I Hypersensitivity reactions:

Type I hypersensitivity occurs as a result of exposure to an antigen. The antigens are proteins with a molecular weight ranging from 10 to 40 kDa. These reactions mediated by IgE antibodies may occur upon first exposure to a substance when prior sensitization to homologous proteins from other sources has occurred. This cross-reactivity explains the onset of symptoms even without previous direct contact.

Types of antigens involved

- Food: nuts, eggs, soy, wheat, shellfish, etc.
- Animal source: bees, wasp, cats, insects, rats, etc.
- Environmental factors: dust mites, latex, pollen, mold, flowers smell, etc.
- Atopic diseases: allergic asthma, allergic rhinitis, conjunctivitis, dermatitis, etc.

Type II hypersensitivity reaction

Type II reactions refers to which antibodies (IgG or IgM) are directed against cellular or extracellular matrix antigens with the resultant cellular destruction, functional loss, or damage to tissues.

The antigens may be for example, on the cell membrane of erythrocytes that are key molecules that determine blood types. Depending on the chemical nature of the antigens, blood types have different levels of hypersensitivity; for instance, A and B are more antigenic than other antigens.Damage can be accomplished via three different mechanisms:

- Antibody binding to cell surface receptors and altering its activity
- Activation of the complement pathway.
- Antibody-dependent cellular cytotoxicity.

Type-III hypersensitivity:

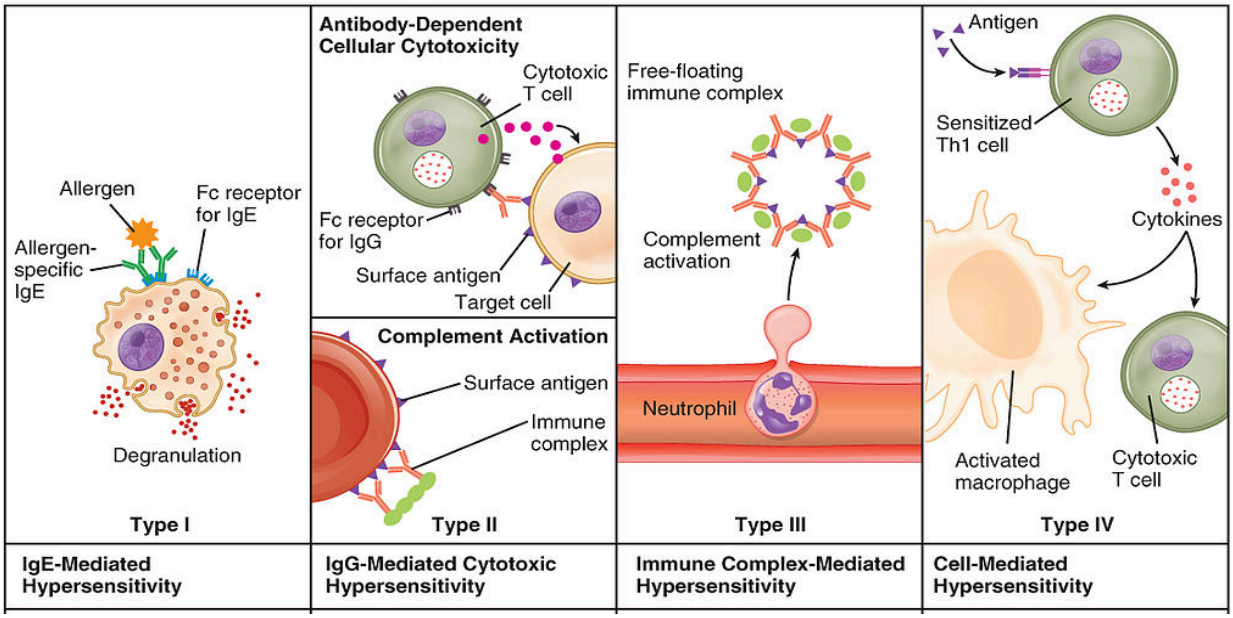
In type III hypersensitivity reaction, an abnormal immune response is mediated by the formation of antigen-antibody aggregates called "immune complexes". They can precipitate in various tissues such as skin, joints, vessels, or glomeruli, and trigger the classical complement pathway. Complement activation leads to the recruitment of inflammatory cells (monocytes and neutrophils) that release lysosomal enzymes and free radicals at the site of immune complexes, causing tissue damage.

The most common diseases involving a type III hypersensitivity reaction are serum sickness, post-streptococcal glomerulonephritis, systemic lupus erythematosus, farmers' lung (hypersensitivity pneumonitis), and rheumatoid arthritis

Type-IV hypersensitivity:

A type IV hypersensitivity reaction is mediated by T cells that provoke an inflammatory reaction [21]against exogenous or endogenous antigens. In certain situations, other cells, such as monocytes, eosinophils, and neutrophils, can be involved. After antigen exposure, an initial local immune and inflammatory response occurs that attracts leukocytes. The antigen engulfed by the macrophages and monocytes is presented to T cells, which then becomes sensitized and activated. These cells then release cytokines and chemokines, which can cause tissue damage and may result in illnesses.[9]

Examples of illnesses resulting from type IV hypersensitivity reactions include contact dermatitis and drug hypersensitivity.

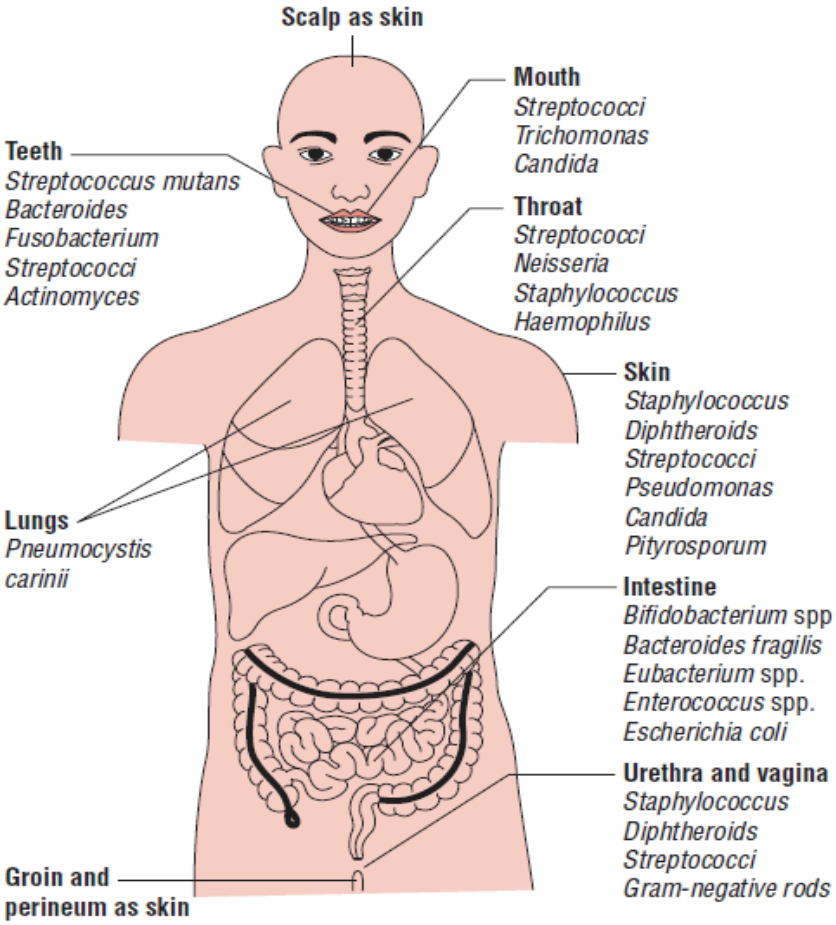


Unit-3:

1. **Review about the normal flora present on the human body**

The human body is exposed to microorganisms in the environment every day. Hundreds of species and countless individual microbial cells, collectively called the **normal microflora**, grow on or in the human body. This is also called the **human microbiome**, the sum total of all

microorganisms that live on or in the human body. These microorganisms can be of two types. Resident flora and transient flora.



Normal Microbiota of the Skin

The resident flora consists of relatively fixed types of microorganisms regularly found in a given area at a given age; if disturbed, it promptly reestablishes itself. Resident flora of certain areas plays a definite role in maintaining health and normal function. Members of the resident flora in the intestinal tract synthesize vitamin K and helps in the absorption of nutrients. On mucous membranes and skin, the resident flora may prevent colonization by pathogens and possible disease through "bacterial interference." like competition for receptors or binding sites on host cells, competition for nutrients, mutual inhibition by metabolic or toxic products, mutual inhibition by antibiotic materials or bacteriocins, or other mechanisms.

The transient flora consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks; it is derived from the environment, does not produce disease, and does not establish itself permanently on the surface.

Normal flora of Conjunctiva: The conjunctiva is relatively free from bacteria due to the presence of lysozyme in the tears which flushes the bacteria. Predominant organisms of the eyes are:Moraxella sp, Diphtheroids , Straph epidermidis, Moraxella sp , Non hemolytic streptococci

Normal Flora of Nose and Nasopharynx

The nasopharynx of the infant is sterile at birth but in 2-3 days time it acquires the flora carried by the mother and attendants. The nasopharynx is a natural habitat of the common pathogenic bacteria causing infection of the nose, throat, bronchi and lungs. The flora of nose harbours Diptheroids, Straphylococcus, Streptococcus, Haemophilus, and Moraxella lacunata .

Normal Flora of the Mouth

The mouth contains micrococci, gram positive aerobic spore bearing bacilli, coliforms, proteus and lactobacilli. The gums pockets between the teeth and crypts of the tonsils have a wide spectrum of anaerobic flora like fusiform bacilli, treponemes, lactobacilli, etc. Candida is also found.

The mouth of infant is not sterile at birth. It generally contains the same types of organisms as found in mother's vagina. These bacteria diminish in number and are replaced by similar bacteria present in the mouth of mother and nurse.

Normal Flora of Upper Respiratory Tract

Within 12 hours of birth alpha hemolytic streptococci are found in upper respiratory tract and become the dominant organism of the oropharynx and remain for the whole life. In the pharynx and trachea, similar flora is established. Smaller bronchi and alveoli are normally sterile.

Normal Flora of Gastrointestinal Tract:

The GI Tract of the fetus in utero is sterile. It becomes contaminated with organisms shortly after birth. In breast fed infants, the intestine contains lactobacilli, enterococci, colon bacilli and staphylococci.

In bottle fed infants the intestine contains anaerobic lactobacilli, colon bacilli and aerobic and anaerobic spore bearing organisms. With the change of food, flora changes. Diet has a marked influence on the composition of the intestinal and fecal flora.

In the stomach as pH is low, the stomach is sterile but as the pH increases in small intestine the number of bacteria increases progressively beyond the duodenum to the colon. The bacterial count is low in small intestine as compared to large intestine. Lactobacilli and entrococci predominate in the duodenum and proximal ileum. The bacterial flora is similar in lower ileum, caecum and rectum. The anaerobic condition of colon is maintained by aerobic bacteria which utilizes the free oxygen.

Normal Flora of the Genitourinary Tract

Mycobacterium smegmatis a harmless commensal is found in the secretions (smegma) of both males and females genitalia. They may pose the confusion with the tubercle bacilli. Strains of

mycoplasma and ureaplasma are frequently present as part of normal flora. Gardnerella vaginalis, bacteroides and alpha streptococci have been found in penile urethra.
Female urethra is either sterile or contains staphylococcus epidermidis. The vagina of newly borne child is sterile and within 24 hours it colonizes with micrococci, entrococci. In 2-3 days time doderlien's bacillus appears. So the flora keeps on changing depending upon the pH of the vagina. Doderlien bacilli remain in the vagina till menopause. After menopause flora resembles that before puberty.

2. **Generalise Opportunistic and Nosocomial infections**

Opportunistic infections (OIs) are infections that take advantage of a weakened immune system. They occur when pathogens, which normally don't cause illness, or common pathogens in unusually severe forms, infect individuals with compromised immune defenses. This can happen due to conditions like HIV, cancer treatment, or organ transplantation.

Weakened Immune System:
OIs are more likely to occur in people with impaired immune systems, making them susceptible to infections that wouldn't typically affect healthy individuals.

Pathogens:
OIs can be caused by various pathogens, including bacteria, fungi, viruses, and parasites.

Severity:
Some OIs can be more severe or occur more frequently in individuals with weakened immune systems.

Examples:
Some common OIs include candidiasis, toxoplasmosis, and tuberculosis.

Prevention and Treatment:
Maintaining a strong immune system (e.g., through HIV medication adherence), and taking preventative medications can help reduce the risk of OIs.

Nosocomial infections.

A nosocomial infection are commonly known as a healthcare-associated infection (HAI), is an infection acquired in a healthcare facility (like a hospital or clinic) that was not present when the patient was admitted. These infections are caused by pathogens such as bacteria, viruses, or fungi, and can spread through contaminated equipment, surfaces, air, and healthcare workers. Common types include urinary tract infections (UTIs), pneumonia, and surgical site infections, and they pose a significant risk to vulnerable patients. Prevention focuses on diligent hand hygiene, proper equipment disinfection, and careful management of medical devices.

Definition:
An infection that develops in a healthcare setting but was not incubating or present when the patient was admitted.

Common Locations:

Hospitals, nursing homes, outpatient clinics, and even after discharge from a facility.

Causes:

Transmission of pathogens (germs) from other infected individuals, the environment (e.g., contaminated water, surfaces), or even from a patient's own bacteria that become opportunistic.

Common Types and Risk Factors

Common Types:Urinary tract infections (UTIs), surgical site infections, pneumonia (respiratory infections), and wound infections are frequently seen, according to the World Health Organization (WHO).

Risk Factors:Invasive medical procedures and surgeries

Use of indwelling devices like urinary catheters or prosthetic implants

Weakened immune systems

Prolonged hospital stays

Advanced age

Prevention and Control

- Hand Hygiene: Frequent handwashing by healthcare workers is the most critical step to prevent the spread of germs.
- Aseptic Techniques: Practicing proper sterile techniques during procedures and wound care.
- Device Management: Minimizing the use of catheters and other invasive devices and removing them as soon as medically appropriate.
- Protective Measures: Wearing personal protective equipment (PPE) such as gloves and face masks.

3.Give a general account on causal organism, pathogenesis , diagnosis and prevention of Tuberculosis

Tuberculosis (TB) is a bacterial infection, primarily caused by Mycobacterium tuberculosis, that mainly affects the lungs but can impact any organ. Spread through airborne droplets from people with active pulmonary TB, it presents as a latent infection (no symptoms, non-contagious) or active disease, which requires a long course of antibiotics for treatment.

Morphology of Mycobacterium tuberculosis

1. Straight or slightly curved thin rod-shaped bacilli.
2. Non-sporing, non-motile, non-capsulated bacteria.
3. Acid-fast bacilli, neither gram +ve nor gram –ve.
4. During acid-fast stain, they appear bright red to intensive purple with green/blue background.
5. They measure 0.5 μm x 3 μm.
6. Appears in single, in pairs, or small chumps.
7. The high content of mycolic acid (50 to 60 %).
8. The cell wall is rich in lipids and waxes.
9. They are wrapped together due to the presence of fatty acids.
10. Capable of intracellular growth.
11. They are resistant to disinfectants, detergents, common antibiotics, dyes, stains, osmotic lysis, lethal oxidation, etc.

Culture characters:In the Lowenstein-Jensen (LJ) medium

1. It is an egg-based medium and growth is quite slow.
2. It takes 6-8 weeks to get visual colonies on this type of media.
3. Colonies are non-pigmented, dry, rough, raised, irregular with a wrinkled surface
4. They are creamy-white initially, becoming yellowish or buff-colored on further incubation.
5. Growth is eugonic (grows more luxuriantly in culture).
6. The optimum temperature is 35-37°C and the optimum pH is 6.4 to 7.
7. A faster result can now be obtained using the Middlebrook medium or BACTEC.
8. It is an obligate aerobe.

Pathogenesis

1. Portal of entry

- The infectious bacilli gain its entry to the human host from inhalation as droplets from the atmosphere.
- The first line of defense of the host tries to ward off the bacteria by tracheal and bronchial epithelium.
- When bacteria survive from the first line of defense, they enter the lungs.

2. Primary tuberculosis

- The bacteria are phagocytosed by alveolar macrophages in the lung, entraps the bacteria in phagosome and action of lysosome along with phagosome collectively called as phagolysosome to kill the bacteria.
- When the bacteria survive the initial host defense mechanism, it attacks alveolar macrophages, gains access to the lung parenchyma, survives in the phagosome, and induces a localized cell-mediated pro-inflammatory response leading to granuloma formation.
- The granuloma protects the bacteria and generally appear after 3 weeks of primary infection.
- When fully developed, this lesion, a chronic granuloma, consists of three zones
    1. a central area of large, multinucleated giant cells containing tubercle bacilli;
    2. a mid-zone of pale epithelioid cells often arranged radially; and
    3. a peripheral zone of fibroblasts, lymphocytes, and monocytes.
- The center of the granuloma contains a mixture of necrotic tissue and dead macrophages.
- Being metabolically very active, the macrophages in the granuloma consume oxygen, and the resulting anoxia and acidosis in the center of the lesion probably kill most of the bacilli.
- Granuloma formation is usually sufficient to limit the primary infection.
- The lesions become quiescent and surrounding fibroblasts produce dense scar tissue, which may become calcified called ghon focus.
- Programmed cell death (apoptosis) of bacteria-laden macrophages by cytotoxic T cells and natural killer (NK) cells contributes to protective immunity by generating a metabolic burst that kills tubercle bacilli.

Laboratory diagnosis :: Specimen and processing

- sputum, bronchial washings, brushings or biopsies or early morning gastric aspirates, Cerebrospinal Fluid (CSF), urine

Direct detection methods

1. Microscopy

- Detection of the acid-fast property of mycobacteria to detect them in sputum and other clinical material by the Ziehl–Neelsen (ZN) staining technique.
- Red bacilli are seen against the contrasting background color.
- Slender, straight, or slightly curved rods with a barrel or beaded appearance.
- Fluorescence microscopy, based on the same principle of acid-fastness, is increasingly used and is much less tiring.

2. Culture

- As sputum and certain other specimens frequently contain many bacteria and fungi that would rapidly overgrow any mycobacteria are decontaminated by treating with 10% NaOH and the deposit is used to inoculate the Lowenstein Jensen (LJ) medium.
- Specimens such as cerebrospinal fluid and tissue biopsies, which are unlikely to be contaminated, are inoculated directly onto culture media.
- Dry, rough, raised, wrinkled, off white to buff-colored colonies on LJ medium, commonly called as rough, tough, and buff colonies.

3. Biochemical analysis

- Niacin accumulation test: positive
- Arylsulphatase test: positive
- Neutral red test: positive
- Catalase peroxidase test: peroxidase positive and weakly catalase-positive
- Amidase test: positive
- Nitrate reduction test: positive

5. Serodiagnosis

- ELISA techniques have been attempted for rapid diagnosis of different clinical forms of tuberculosis by estimating specific IgM, IgA, and IgG antibody titers employing the use of various mycobacterial antigens.

7. Molecular techniques

- Subsequent to the introduction of commercially available hybridization assays, commercially available and inhouse– developed nucleic acid amplification tests were used successfully for early identification of *M. tuberculosis* complex grown in liquid cultures.
- PCR-based sequencing for mycobacterial identification consists of PCR amplification of mycobacterial DNA with genus-specific primers and sequencing of the amplicons.

Indirect detection methods: Tuberculin test

- A purified protein derivative (PPD) is obtained by chemical fractionation of old tuberculin.
- PPD is standardized in terms of its biologic reactivity as tuberculin units (TU).
- A large amount of tuberculin injected into a hypersensitive host may give rise to severe local reactions and a flare-up of inflammation and necrosis at the main sites of infection (focal reactions).

- For this reason, tuberculin tests in surveys use 5 TU in 0.1 mL solution; in persons suspected of extreme hypersensitivity.
- After the tuberculin skin test is placed, the area is examined for the presence of induration no later than 72 hours after placement.
- For persons at low risk for tuberculosis, 15 mm or larger of induration is considered a positive test result.
- Positive test results tend to persist for several days. Weak reactions may disappear more rapidly.

Treatment: The first line of anti-TB agents that form the core of treatment regimens are

- Isoniazid (INH)
- Rifampin (RIF)
- Pyrazinamide (PZA)
- Ethambutol (EMB)

The second line drugs include

- A later generation of fluoroquinolones such as moxifloxacin, levofloxacin, or gatifloxacin.
- An injectable agent such as amikacin, kanamycin, or capreomycin.
- Two or more core second-line agents include ethionamide, prothionamide, cycloserine, terizidone, clofazimine, or linezolid.

Prevention: Human tuberculosis is preventable:

- by the early detection and effective therapy of the open or infectious individuals in a community
- by lowering the chance of infection by reducing overcrowding as the most important factors affecting the incidence of tuberculosis are socio-economic ones, particularly those leading to a reduction of overcrowding in homes and workplaces.
- to a limited extent, by vaccination which includes BCG vaccination that is given by intracutaneous injection after the birth.
- neonatal vaccination is recommended as prior exposure of the human population to environmental mycobacteria confer some protection, but in others induce inappropriate immune reactions that antagonize protection.

4. Write about Diagnosis and prevention of Hepatitis A and AIDS

Hepatitis A is an inflammation of the liver that can cause mild to severe illness. The hepatitis A virus (HAV) is transmitted through ingestion of contaminated food and water or through direct

contact with an infectious person. Almost everyone recovers fully from hepatitis A with a lifelong immunity. However, a very small proportion of people infected with hepatitis A could die from fulminant hepatitis.

- The risk of hepatitis A infection is associated with a lack of safe water and poor sanitation and hygiene (such as contaminated and dirty hands). A safe and effective vaccine is available to prevent hepatitis A.

Diagnosis of Hepatitis A

- Clinical Symptoms
  Fever, fatigue, loss of appetite, nausea, vomiting.
  Jaundice (yellowing of skin/eyes), dark urine, pale stools.

- Laboratory Tests
  Serological Test:
  Detection of anti-HAV IgM antibodies → confirms *acute infection*.
  Presence of anti-HAV IgG antibodies → indicates *past infection or immunity*.
  Liver function tests (LFTs): Elevated ALT, AST, and bilirubin levels.

Prevention of Hepatitis A

1. General Measures
   Ensure safe drinking water.
   Proper sanitation and personal hygiene (handwashing with soap).
   Avoid consumption of raw/undercooked shellfish and contaminated food.

2. Immunization
   Hepatitis A vaccine: Highly effective, recommended for children (≥1 year), travelers to endemic areas, and high-risk groups.
   Post-exposure prophylaxis: Hepatitis A vaccine or immune globulin (within 2 weeks of exposure).

3. Health Education
   Awareness about transmission (fecal–oral route).
   Promotion of hygienic food-handling practices.

HIV & AIDS

Human immunodeficiency virus (HIV) is a virus that attacks the body's immune system. Acquired immunodeficiency syndrome (AIDS) occurs at the most advanced stage of infection. HIV targets the body's white blood cells, weakening the immune system. This makes it easier to get sick with diseases like tuberculosis, infections and some cancers.

HIV is spread from the body fluids of an infected person, including blood, breast milk, semen and vaginal fluids. It is not spread by kisses, hugs or sharing food. It can also spread from a mother to her baby.HIV can be prevented and treated with antiretroviral therapy (ART). Untreated HIV can progress to AIDS, often after many years.

Diagnosis of AIDS

- **Screening Tests**:
  ELISA (Enzyme-Linked Immunosorbent Assay) – detects antibodies to HIV.
  Rapid antibody tests – quick detection, often used for mass screening.

- **Confirmatory Test**:
  Western Blot / Immunoblot – confirms HIV infection after a positive screening test.

- **Direct Detection**:
  PCR (Polymerase Chain Reaction) – detects viral RNA/DNA, used in early infection and monitoring viral load.
  p24 Antigen Test – detects HIV core protein, useful in early infection before antibodies appear.

- **Monitoring of Disease Progression**:
  CD4+ T-cell count – assesses immune system status.
  Viral load testing – measures amount of virus in blood.

Prevention of AIDS

- **Safe Sexual Practices**: Use of condoms, avoiding multiple partners, and promoting sexual health education.
  Blood Safety: Screening of blood and blood products for HIV before transfusion.
  Safe Injection Practices: Avoid sharing of needles, use sterile syringes.
  Prevention of Mother-to-Child Transmission: Antiretroviral therapy (ART) during pregnancy, safe delivery practices, and avoidance of breastfeeding when alternatives are safe.

Health Education & Awareness: Spreading knowledge about modes of transmission and dispelling myths.

Antiretroviral Therapy (ART): Early initiation reduces transmission risk and improves quality of life.

Preventive Drugs:

Pre-exposure prophylaxis (PrEP) for high-risk individuals.

Post-exposure prophylaxis (PEP) within 72 hours of possible exposure.

Un**it-4**

1. **Explain the process of collection and handling and processing of clinical samples**

The collection, handling, and processing of clinical samples is a critical component of diagnostic microbiology and laboratory medicine. The accuracy of laboratory results, and consequently the correct diagnosis and management of patients, depends largely on the quality of the specimen received. A poorly collected or improperly handled sample can lead to false results, misdiagnosis, and inappropriate treatment. Hence, strict adherence to standard protocols is essential.

Collection of Clinical Samples

The collection of samples must always be carried out using aseptic precautions to prevent contamination with commensal flora or environmental organisms. It is important to collect specimens before the administration of antimicrobial therapy whenever possible. The specimen should be adequate in quantity, representative of the diseased site, and collected into sterile, leak-proof containers.

- Blood: Collected using sterile venipuncture techniques. For blood cultures, multiple sets from different venipuncture sites are required to improve diagnostic yield.
- Urine: The preferred specimen is a clean-catch midstream urine. In infants, catheterization or suprapubic aspiration may be performed.
- Sputum: Early morning deep cough specimens should be collected to avoid contamination with saliva.

- Throat and nasal swabs: Collected with sterile swabs, taking care not to touch surrounding mucosa.
- Stool: Freshly passed stool is collected in clean containers; contamination with urine must be avoided.
- Cerebrospinal fluid (CSF): Obtained by lumbar puncture in sterile tubes and must be processed immediately.
- Pus and wound material: Aspirated pus is preferred over swabs, as it reduces contamination and improves diagnostic accuracy.

Each specimen should be clearly labeled with the patient's identification, date, time, and type of specimen, and accompanied by a requisition form containing clinical details.

Handling of Clinical Samples

Once collected, specimens must be handled with care to preserve the viability of pathogens and prevent overgrowth of contaminants. Transport should be as rapid as possible to the laboratory. If delays are expected, appropriate transport media should be used:

- Stuart's or Amies medium for throat and wound swabs.
- Cary-Blair medium for stool specimens.
- Viral transport medium (VTM) for viral samples.

Temperature is also crucial: most specimens may be kept at 4°C for short periods, but certain samples like CSF should never be refrigerated and must be processed immediately. Specimens should always be transported in a leak-proof container with appropriate biohazard labeling to ensure the safety of healthcare and laboratory staff.

Processing of Clinical Samples

In the laboratory, samples are first examined for labeling, container integrity, and adequacy. Processing includes several steps:

1. Macroscopic Examination: Assessing color, consistency, presence of blood or pus.

2. Microscopic Examination: Direct smears are prepared for Gram staining, acid-fast staining, or wet mount preparations. This provides rapid preliminary information.

3. Culture: Inoculation onto suitable media under sterile conditions, followed by incubation under appropriate conditions (aerobic, anaerobic, or CO_2 enriched environments).

4. Identification: Based on colony morphology, biochemical reactions, serological tests, or modern molecular techniques such as PCR.

5. Antimicrobial Susceptibility Testing: To guide clinicians in choosing effective therapy.

Precautions and Biosafety

Specimen collection and processing involve potential exposure to infectious agents. Therefore, strict biosafety precautions must be followed. Personal protective equipment (PPE), use of biosafety cabinets for high-risk pathogens, and safe disposal of biomedical waste are essential. Proper communication between clinicians and laboratory personnel further ensures accuracy and safety.

2. Describe different methods of identification by culturing of clinical samples

Introduction

When a clinical specimen such as blood, urine, sputum, pus, or cerebrospinal fluid is received in the laboratory, one of the primary steps in processing is inoculation onto appropriate culture media. The goal is to recover the causative pathogen, distinguish it from commensal or contaminant organisms, and identify it accurately for guiding treatment.

Steps in Identification by Culture

1. Inoculation onto Media

- The sample is inoculated onto different culture media using aseptic techniques.
- The choice of media depends on the suspected pathogen and the type of specimen.
    - Nutrient agar / Blood agar – for most bacteria.
    - MacConkey agar – for Gram-negative enteric bacilli.
    - Lowenstein-Jensen medium – for *Mycobacterium tuberculosis*.
    - Sabouraud's agar – for fungi.

- Selective and differential media may be used to suppress unwanted organisms and differentiate between species.

2. Incubation

- Inoculated media are incubated at appropriate temperatures and atmospheric conditions:
  35–37°C for most bacteria.
  25–30°C for fungi.
  Special conditions like anaerobic jars for anaerobes, or CO_2 incubators for fastidious organisms.

3. Observation of Growth (Colony Morphology)

- Colonies are observed for:
  Size, shape, elevation, margin, surface texture.
  Color, opacity, consistency, and hemolysis on blood agar.

- These colony characteristics provide important preliminary clues for identification.

4. Microscopic Examination

Smears are prepared from colonies and stained (Gram stain, Ziehl-Neelsen, India ink, etc.).
Microscopy provides information about Gram reaction, shape, arrangement, and presence of special structures (spores, capsules, flagella).

5. Biochemical Tests

- Isolated colonies are subjected to biochemical reactions to confirm identity.
    - Catalase, oxidase, coagulase for staphylococci and related organisms.
    - Sugar fermentation, indole, methyl red, Voges-Proskauer, citrate, urease for enteric bacteria.
      Automated systems (VITEK, API strips) are widely used for rapid biochemical profiling.

6. Serological Methods

- Some bacteria are identified by agglutination or antigen detection after culture.
  e.g., *Salmonella* and *Shigella* are confirmed by serotyping.

7. Antimicrobial Susceptibility Testing (AST)

- Once an organism is identified, susceptibility to antibiotics is determined by:
  Disk diffusion (Kirby-Bauer method).
  Broth dilution (MIC determination).
  Automated systems.
  SUMMARY:

Step	Method/Approach	Examples / Notes
1. Inoculation	Clinical specimen inoculated onto suitable media	Nutrient agar, Blood agar, MacConkey agar, Chocolate agar, Sabouraud's agar, Lowenstein-Jensen medium
2. Incubation	At appropriate temperature and atmosphere	35–37°C (most bacteria), 25–30°C (fungi), anaerobic jars, CO_2 incubators
3. Colony Morphology	Observe size, shape, elevation, color, opacity, hemolysis	*Staphylococcus aureus*: golden yellow colonies; *E. coli*: lactose-fermenting pink colonies on MacConkey agar
4. Microscopy from Colony	Gram stain, Ziehl-Neelsen, India ink, wet mount	Gram-positive cocci in clusters (*Staphylococcus*); acid-fast bacilli (*Mycobacterium*)
5. Biochemical Tests	Carbohydrate fermentation, enzyme tests	Catalase, Oxidase, Coagulase (Staphylococcus); IMViC (Enterobacteriaceae); Urease (Proteus)
6. Serological Confirmation	Agglutination / antigen detection after culture	*Salmonella* and *Shigella* serotyping
7. Antimicrobial Susceptibility Testing (AST)	Determines sensitivity to antibiotics	Disk diffusion (Kirby-Bauer), MIC by broth dilution, Automated systems (VITEK, Phoenix)

3. Demonstrate process of PCR and DNA probes in identification of pathogen

Molecular diagnostic methods have revolutionized the identification of infectious agents. Among these, **Polymerase Chain Reaction (PCR)** and **DNA probes** are the most widely used tools. These techniques are highly sensitive, specific, and rapid compared to conventional culture methods, and they allow direct detection of pathogens from clinical specimens.

1. Polymerase Chain Reaction (PCR)

PCR is a molecular technique that amplifies a specific segment of DNA, enabling detection of even a few copies of microbial nucleic acids in a clinical specimen.

Steps in PCR Process:

1. **Sample Preparation:**
   DNA or RNA is extracted from the clinical specimen (e.g., blood, sputum, CSF).
   For RNA viruses, reverse transcriptase is used to convert RNA into complementary DNA (cDNA).

2. **Denaturation (95°C):**
   Double-stranded DNA is heated to separate into single strands.

3. **Annealing (50–65°C):**
   Short synthetic primers bind (anneal) to the complementary sequences on target DNA.

4. **Extension (72°C):**
   DNA polymerase (usually Taq polymerase) extends the primers, synthesizing new DNA strands.

5. **Amplification Cycle:**
   These three steps are repeated for 25–40 cycles in a thermocycler, producing millions of copies of the target DNA.

6. **Detection:**
   Amplified DNA is detected using gel electrophoresis, fluorescence dyes (SYBR Green), or probes in real-time PCR.

Applications in Pathogen Identification:

Detection of *Mycobacterium tuberculosis* directly from sputum.
Identification of HIV, HBV, HCV, HPV and other viral pathogens.
Differentiation of bacterial strains (e.g., MRSA vs MSSA).

2. DNA Probes

A DNA probe is a **short, single-stranded DNA fragment** labeled with a radioactive isotope, fluorescent dye, or enzyme. It is designed to be complementary to a specific DNA sequence of the pathogen.

Process of DNA Probe Technique:

1. **Preparation of Probe:**

- A specific DNA sequence unique to the pathogen is synthesized and labeled.

2. **Denaturation of Target DNA:**
   Clinical specimen DNA is extracted and denatured into single strands.

3. **Hybridization:**
   The labeled probe is allowed to hybridize (bind) with its complementary sequence in the sample.

4. **Washing:**
   Unbound probes are removed by washing.

5. **Detection:**
   Hybridized probes are detected by autoradiography, fluorescence, or colorimetric reaction depending on the label used.

Applications in Pathogen Identification:

- Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genital specimens.
- Identification of *Mycobacterium tuberculosis* complex.
- Differentiation of bacterial and viral species and strains.

Comparison of PCR and DNA Probes

Aspect	PCR	DNA Probe
Sensitivity	Extremely high (amplifies DNA)	Moderate (no amplification)
Time required	Few hours	Few hours
Specificity	Very high (primer-dependent)	High (probe sequence-specific)
Applications	Direct detection of low-copy pathogens	Confirmation of pathogen identity after culture

Conclusion

PCR and DNA probe technologies have become invaluable in modern clinical microbiology. PCR allows **rapid amplification and detection** of microbial nucleic acids directly from clinical samples, while DNA probes provide **specific hybridization-based identification**. These molecular methods complement traditional culture and biochemical techniques, offering faster and more reliable diagnosis of infectious diseases, which is crucial for early treatment and effective patient care.

4.Illustrate role of serological tests in identification of pathogen

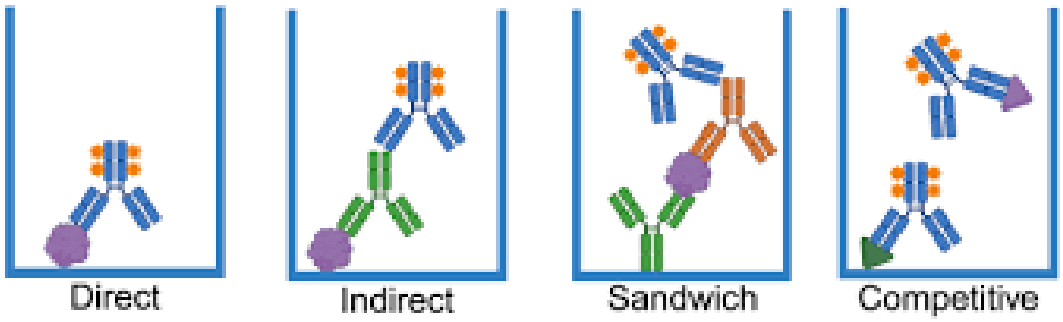
ELISA (Enzyme-Linked Immunosorbent Assay)

Principle

- Based on the **antigen–antibody reaction**.
- Detection is achieved using an **enzyme-labeled antibody**.
- A **chromogenic substrate** reacts with the enzyme, producing a **color change**, which indicates the presence of antigen or antibody.

Types of ELISA

1. **Direct ELISA** – detects antigen using an enzyme-labeled antibody.
2. **Indirect ELISA** – detects antibodies; antigen is coated on plate, followed by primary antibody and enzyme-linked secondary antibody.
3. **Sandwich ELISA** – antigen is captured between two specific antibodies (highly sensitive).
4. **Competitive ELISA** – sample antigen competes with labeled antigen for antibody binding.



Applications

- Diagnosis of **HIV, Hepatitis, Dengue, COVID-19**.

- Detection of **hormones (e.g., hCG in pregnancy tests)**.
- Screening for **allergens, toxins, and drugs**.
- Used in **research and immunology labs** for quantifying proteins.

Advantages

- **Highly sensitive and specific**.
- Can be **quantitative or qualitative**.
- Suitable for **large-scale screening**.

Limitations

- Requires **special reagents and equipment**.
- May give **false positives/negatives** if not carefully controlled.

Immunofluorescence

- **Definition**: A diagnostic technique that uses antibodies labeled with a fluorescent dye (fluorochrome) to detect the presence of specific antigens in tissues or cells under a fluorescence microscope.
- **Principle**: Antibody–antigen interaction is visualized because the fluorescent dye emits visible light when exposed to UV light.
- **Types**:
    - **Direct immunofluorescence** – Fluorescent dye is directly attached to the antibody that binds to the antigen.
    - **Indirect immunofluorescence** – An unlabeled primary antibody binds to the antigen, and a fluorescent-labeled secondary antibody binds to the primary antibody (more sensitive).
- **Applications**:
    - Detection of pathogens (e.g., viruses, bacteria, parasites).
    - Diagnosis of autoimmune diseases (e.g., SLE, pemphigus).
    - Research in cell biology to study protein localization.

- **Examples**:

1. Detection of rabies virus in brain tissue.
   2. ANA test for autoimmune disorders.

Agglutination Test

Definition: A serological test in which **antigen–antibody reaction** is detected by visible clumping (agglutination) of particles such as red blood cells, bacteria, or latex beads.

Principle: When specific antibodies bind to particulate antigens, cross-linking occurs, resulting in visible clumps.
Types:

Slide agglutination – Performed on a slide, gives rapid results (e.g., Widal test for typhoid).
Tube agglutination – Quantitative, performed in serial dilutions.
Passive (indirect) agglutination – Soluble antigens are attached to carrier particles (e.g., latex, RBCs).
Reverse passive agglutination – Antibodies are coated on particles to detect antigens.

- **Applications**:
  Diagnosis of infectious diseases (e.g., typhoid, brucellosis).
  Blood grouping (ABO, Rh typing).
  Detection of microbial antigens using latex agglutination.

- **Examples**:
  Widal test (Salmonella).
  Latex agglutination for Cryptococcus antigen.

Complement Fixation Test (CFT)

- **Definition**: A classical serological test that detects **antigen–antibody reactions** by using the complement system.

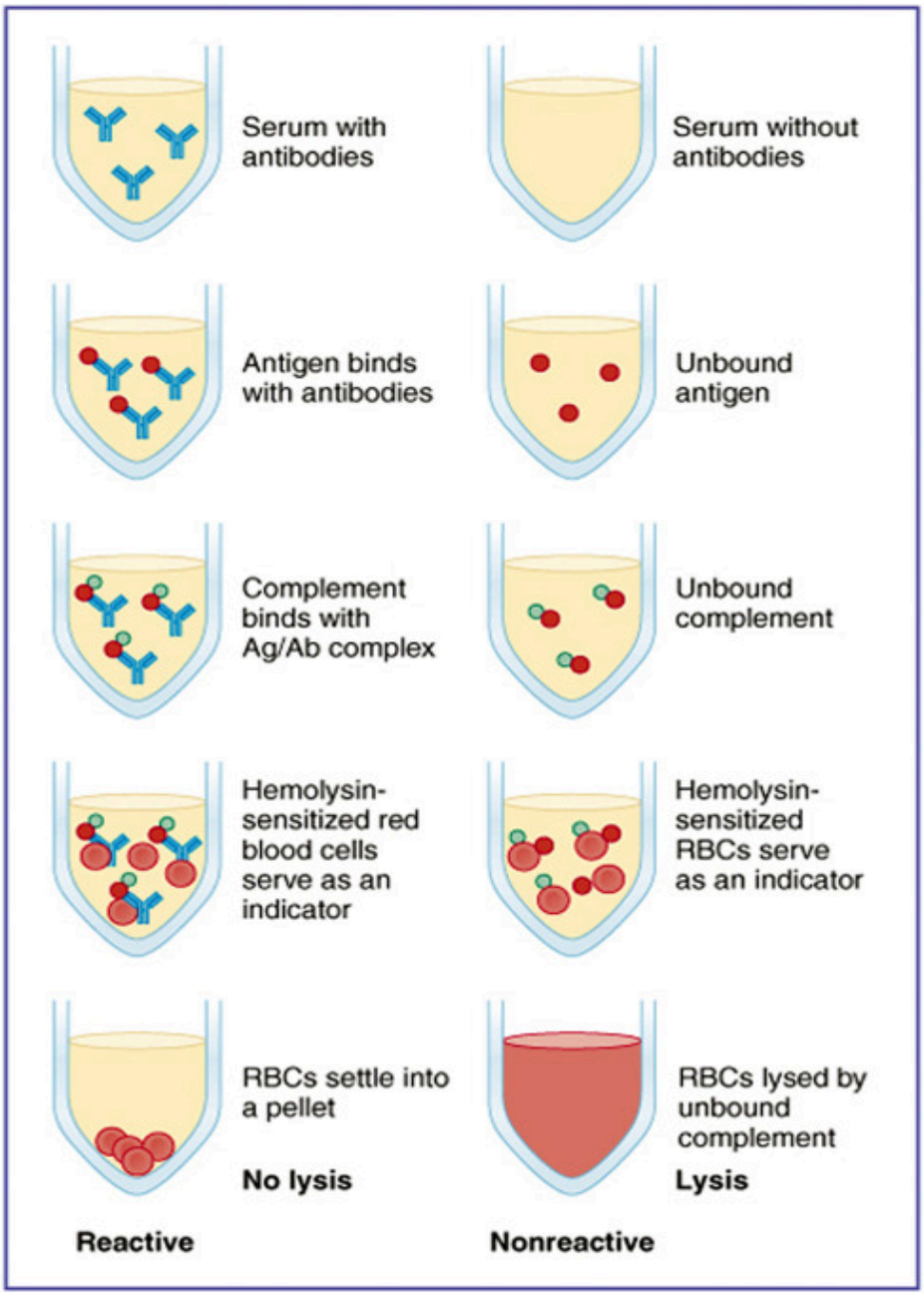
- **Principle**:Complement gets "fixed" (consumed) when antigen–antibody complexes form.
  If complement is fixed, it will not be available to lyse sensitized red blood cells added later → **no hemolysis = positive test**.
  If antigen–antibody reaction does not occur, complement remains free and lyses red blood cells → **hemolysis = negative test**.

- **Steps**:
  Patient's serum (inactivated to remove natural complement) is mixed with specific antigen.

- Complement is added.
  Indicator system (sheep RBCs coated with anti-sheep RBC antibodies) is added.

- **Applications**:
  Diagnosis of viral, bacterial, and parasitic infections (historically used for syphilis, influenza, etc.).
  Detection of antibodies that do not agglutinate or precipitate easily.

- **Limitations**:: Less sensitive than modern methods (ELISA, PCR).Labor-intensive, requires fresh complement.



Unit-V:

1. **Generalise types of vaccines**

Types of Vaccines

Vaccines are biological preparations that provide immunity against specific infectious diseases. They stimulate the immune system to recognize and remember pathogens, thereby preventing future infections. Vaccines can be broadly classified based on the type of antigen used and the method of preparation.

1. Live Attenuated Vaccines

- These vaccines contain living organisms (bacteria or viruses) that have been weakened (attenuated) so they cannot cause disease in healthy individuals.
- They closely mimic natural infections and provide long-lasting immunity.
- **Examples:** BCG (tuberculosis), Oral polio vaccine (OPV), MMR (measles, mumps, rubella), Yellow fever vaccine.
- **Advantages:** Strong and long-lasting immunity.
- **Disadvantages:** Not safe for immunocompromised individuals.

2. Inactivated (Killed) Vaccines

- These vaccines contain microorganisms that have been killed by heat, chemicals, or radiation.
- They are safer than live vaccines but usually require booster doses.
- **Examples:** Inactivated polio vaccine (IPV – Salk), Rabies vaccine, Hepatitis A vaccine.
- **Advantages:** Safe for immunocompromised persons.
- **Disadvantages:** Immunity is weaker and requires multiple doses.

3. Subunit Vaccines

- Instead of the whole microorganism, only specific antigenic parts (proteins, polysaccharides, or conjugates) are used.They are very safe and have fewer side effects.
- **Examples:** Hepatitis B vaccine (recombinant surface antigen), Human papillomavirus (HPV) vaccine.

4. Toxoid Vaccines

- Some diseases are caused by bacterial toxins rather than the bacteria itself. Toxoid vaccines contain inactivated toxins (toxoids) that stimulate immunity.
- **Examples:** Diphtheria toxoid, Tetanus toxoid.
- **Advantages:** Very effective in toxin-mediated diseases.
- **Disadvantages:** Require booster doses.

5. Conjugate Vaccines

- Certain bacteria have polysaccharide capsules that poorly stimulate the immune system. Conjugate vaccines link these polysaccharides to a protein carrier, making them more immunogenic.
- **Examples:** Haemophilus influenzae type b (Hib) vaccine, Pneumococcal conjugate vaccine, Meningococcal vaccine.

6. Recombinant Vector Vaccines

- Harmless viruses or bacteria are genetically engineered to carry genes of a pathogen. These vectors deliver the antigen into the body and stimulate immunity.
- **Examples:** Recombinant adenovirus-based COVID-19 vaccines (e.g., Covishield, Sputnik V).

7. mRNA and DNA Vaccines (New Generation)

- **mRNA vaccines** deliver genetic instructions to host cells to produce the antigen, which then triggers an immune response.

Examples: Pfizer-BioNTech, Moderna COVID-19 vaccines.
DNA vaccines use plasmid DNA to encode antigens. They are still under research for various diseases.

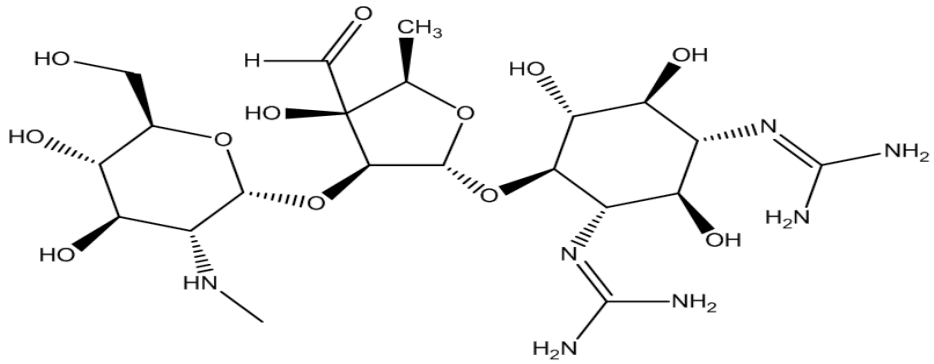
Conclusion

Vaccines are one of the most effective public health tools for controlling infectious diseases. Different types of vaccines—live, killed, subunit, toxoid, conjugate, and newer genetic vaccines—are chosen depending on the nature of the pathogen and safety considerations. With continuous advancements in biotechnology, modern vaccines such as mRNA and recombinant vector vaccines are revolutionizing disease prevention and global health.

2. Explain the mode of action of Pencilline and streptomycine

Antibiotics are powerful chemotherapeutic agents that are used to combat bacterial infections by interfering with vital cellular processes of microorganisms. Among the many antibiotics discovered, Streptomycin and Penicillin are historically significant and widely studied and both are bactericidal in nature; they act on different targets within the bacterial cell.

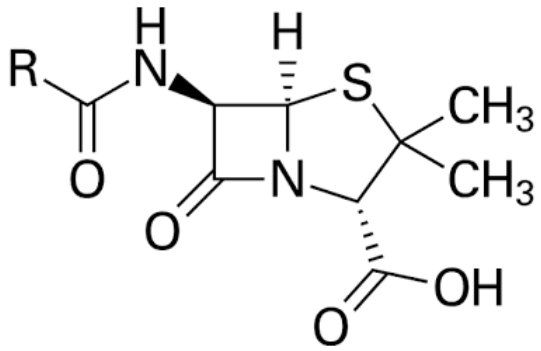
Streptomycin: Mode of Action



Streptomycin is an aminoglycoside antibiotic originally discovered from *Streptomyces griseus*. Its primary target is the bacterial ribosome, the site of protein synthesis. Streptomycin binds irreversibly to the 30S subunit of prokaryotic ribosomes. This binding interferes with the formation of the initiation complex, which is necessary for translation to begin. In addition, the antibiotic causes misreading of the messenger RNA (mRNA) code, leading to the production of nonfunctional or toxic proteins. These faulty proteins disrupt normal cell function and contribute to bacterial cell death.

Because it directly affects protein synthesis, Streptomycin is especially effective against aerobic Gram-negative bacteria and is used in the treatment of diseases such as tuberculosis. Its effect is bactericidal, meaning it kills the bacteria rather than merely inhibiting their growth.

Penicillin: Mode of Action



Penicillin, discovered by Alexander Fleming in 1928, is the first widely used β-lactam antibiotic. Unlike Streptomycin, which targets protein synthesis, Penicillin acts on the bacterial cell wall. Specifically, it inhibits the enzyme transpeptidase (also known as penicillin-binding protein, PBP), which is responsible for the cross-linking of peptidoglycan chains. Peptidoglycan provides structural rigidity to the bacterial cell wall.

When cross-linking is blocked, the cell wall becomes weak and unable to resist osmotic pressure. As a result, bacteria undergo swelling and ultimately cell lysis. Penicillin is most effective against actively growing bacteria, particularly Gram-positive organisms, where the peptidoglycan layer is thick and essential for survival. The action of Penicillin is also bactericidal.

3. Define antibiotic resistance and explain different methods of microbial antibiotic resistance

Antibiotic resistance is the ability of microorganisms such as bacteria to withstand the effects of an antibiotic that would normally kill them or inhibit their growth. This occurs when bacteria undergo genetic changes (mutation or acquisition of resistance genes) or develop protective mechanisms, making the antibiotic ineffective.

Methods of Microbial Antibiotic Resistance

Bacteria can resist the action of antibiotics by several mechanisms. These may occur due to mutation in chromosomal genes or by acquiring resistance genes through plasmids, transposons, or conjugation. The major methods include:

1. Enzymatic Degradation or Modification of the Antibiotic

- Some bacteria produce enzymes that inactivate antibiotics.
- Examples:
    - β-lactamases hydrolyze the β-lactam ring of penicillin and cephalosporins, rendering them inactive.
    - Aminoglycoside-modifying enzymes (acetyltransferases, phosphorylases, adenyltransferases) chemically modify streptomycin, gentamicin, etc

2. Alteration of the Antibiotic Target Site

- Mutations or enzymatic changes alter the binding site of the antibiotic, so the drug can no longer bind effectively.
- Examples:

- Mutation in ribosomal subunits → resistance to streptomycin, erythromycin, tetracycline.
- Alteration of penicillin-binding proteins (PBPs) → resistance to penicillin and methicillin (e.g., MRSA).
- Change in DNA gyrase/topoisomerase → resistance to fluoroquinolones

3. Reduced Permeability of the Cell Membrane

- Bacteria can reduce the uptake of antibiotics by altering their cell wall or outer membrane permeability.
- Example:
  - Changes in porin proteins of Gram-negative bacteria → reduced entry of β-lactams, tetracyclines, and some fluoroquinolones.

4. Active Efflux of Antibiotics

- Bacteria develop efflux pumps that expel antibiotics out of the cell before they reach their target concentration.
- Examples:
  - Efflux pumps for tetracyclines, macrolides, and fluoroquinolones.
  - Multidrug efflux systems in *Pseudomonas aeruginosa*.

5. Bypass Pathways / Metabolic Resistance

- Some bacteria develop alternative metabolic pathways to bypass the action of antibiotics.
- Example:
  - Resistance to sulfonamides: bacteria overproduce PABA or use an alternative pathway for folic acid synthesis, bypassing the drug's inhibitory effect.

Conclusion

Microbes resist antibiotics through mechanisms such as drug inactivation, target modification, reduced drug entry, active efflux, and bypass of metabolic pathways. These strategies enable bacteria to survive even in the presence of antibiotics, making treatment difficult and contributing to the global problem of antimicrobial resistance.

4. Describe the different tests to evaluate antibiotic susceptibility

In the treatment and control of infectious diseases, especially when caused by pathogens that are often drug-resistant, susceptibility (sensitivity) testing is used to select effective antimicrobial drugs. Susceptibility testing is not usually indicated when the susceptibility reactions of a pathogen can be predicted, for example:

– *Proteus* species are generally resistant to nitrofurantoin and tetracyclines,

– *S. pyogenes* is usually susceptible to penicillin,

– *K. pneumoniae* is generally ampicillin-resistant,

– Anaerobes are susceptible to metronidazole.

Laboratory antimicrobial susceptibility testing can be performed using:

– A dilution technique

– A disc diffusion technique.

Dilution susceptibility tests:

Manual or semi-automated dilution susceptibility tests are performed in Microbiology Reference Laboratories for epidemiological purposes or when a patient does not respond to treatment thought to be adequate, relapses while being treated, or when there is immunosuppression.

Dilution techniques measure the minimum inhibitory concentration (MIC). They can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria.

A dilution test is carried out by adding dilutions of an antimicrobial to a broth or agar medium. A standardized inoculum of the test organism is then added. After overnight incubation, the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth.

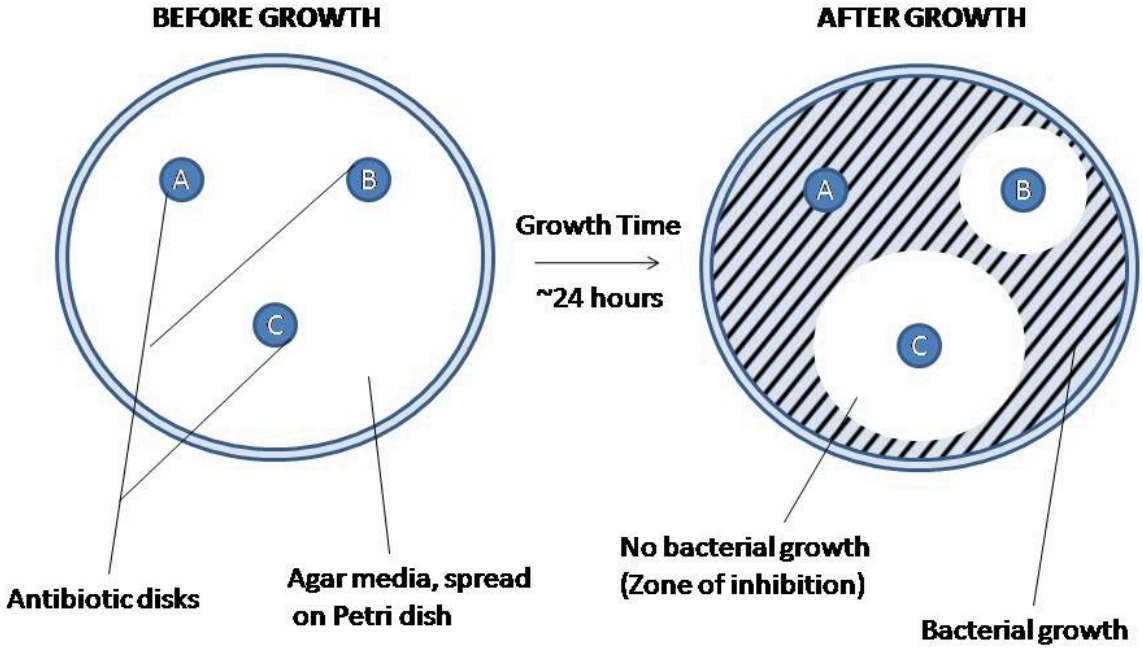
By comparing the MIC value with known concentrations of the drug obtainable in serum or other body fluids, the clinical response can be assessed.

Disc diffusion susceptibility tests

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism.

The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism.

Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc. For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, WHO recommends the (NCCLS- National committee for clinical laboratory standards) modified Kirby-Bauer disc diffusion technique.



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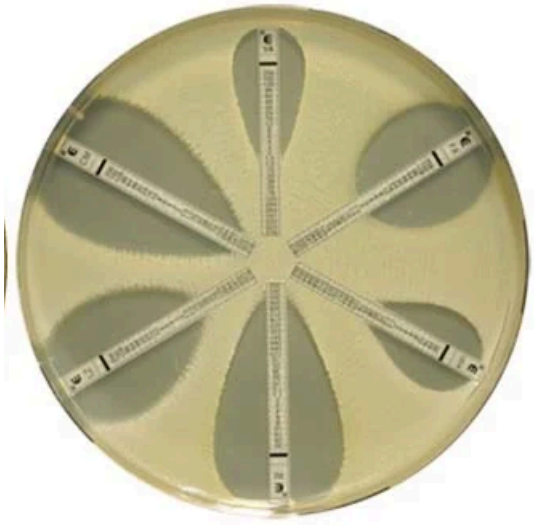
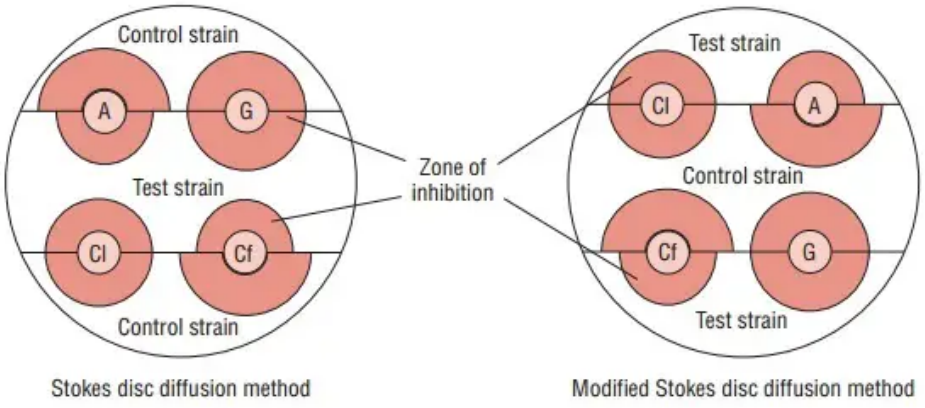


Kirby Bauer NCCLS modified disc diffusion technique:

The validity of this carefully standardized technique depends on, for each defined species, using discs of correct antimicrobial content, an inoculum which gives confluent growth, and a reliable Mueller Hinton agar. The test method must be followed exactly in every detail. After incubation at 35° C for 16–18 hours, zone sizes are measured and interpreted using NCCLS standards. These are derived from the correlation which exists between zone sizes and MICs. The NCCLS Kirby-Bauer technique should only be used for well-evaluated bacterial species. It is not suitable for bacteria that are slow-growing, need special nutrients, or require CO2 or anaerobic incubation.

Stokes disc diffusion technique

In this disc technique, both the test and control organisms are inoculated on the same plate. The zone sizes of the test organism are compared directly with that of the control. This method is not as highly standardized as the Kirby-Bauer technique and is used in laboratories particularly when the exact amount of antimicrobial in a disc cannot be guaranteed due to difficulties in obtaining discs and storing them correctly or when the other conditions required for the Kirby-Bauer technique cannot be met.



Schematic diagram showing Stokes method of antibiotic sensitivity.

Limitations of antimicrobial susceptibility tests

Susceptibility tests measure antimicrobial activity against bacteria under laboratory conditions (in vitro activity), not in the patient (in vivo activity). It cannot be assumed therefore, that an antimicrobial that kills or prevents an organism from growing in vitro will be a successful treatment.