



International Standard **Colored Edition**

2nd
Edition

Textbook of **Microbiology** for GNM Nursing Students *(As per new syllabus of INC for GNM)*



Exclusive Features

- Most updated compendium as per INC new syllabus
- Thoroughly revised and updated edition
- 100+ Illustrations & real-time photographs
- Includes COVID-19 updates and Color Plates on Bacterial, Viral, Fungal diseases, etc.

Edited by
Indarjit Walia
Anju Dhir



CBS Publishers & Distributors Pvt. Ltd.

Mrinalini Bakshi



Nursing Knowledge Tree
An Initiative by CBS Nursing Division

Textbook of **Microbiology** for GNM Nursing Students *(As per new syllabus of INC for GNM)*

Second Edition

Mrinalini Bakshi

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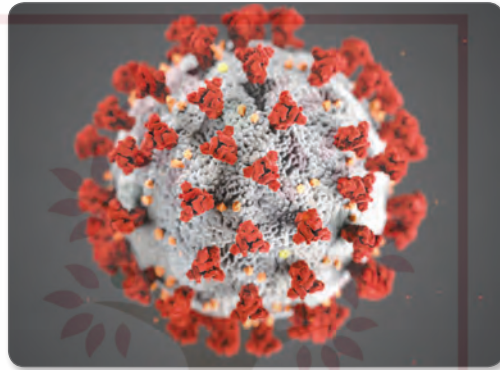


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COVID-19 UPDATES

WHAT IS COVID-19?

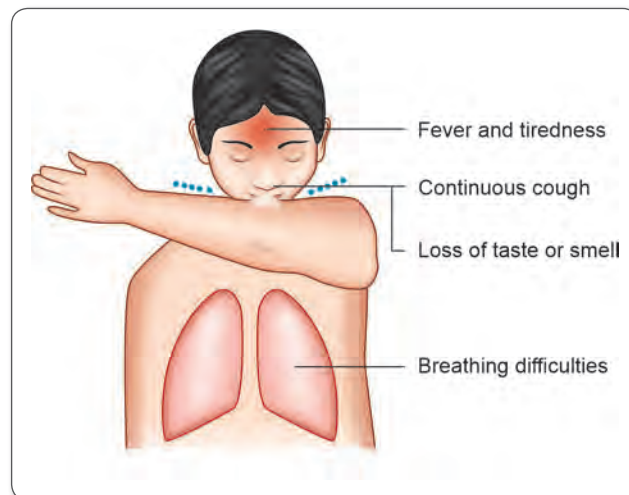
Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus. Most people who fall sick with COVID-19 experience mild to moderate symptoms and recover without special treatment.



HOW IT SPREADS?

- The virus that causes COVID-19 is mainly transmitted through droplets generated when an infected person coughs, sneezes, or exhales. These droplets are too heavy to hang in the air, and quickly fall on floors or surfaces.
- You can be infected by breathing in the virus if you are within reach of someone who has COVID-19, or by touching a contaminated surface and then your eyes, nose or mouth.

SYMPTOMS



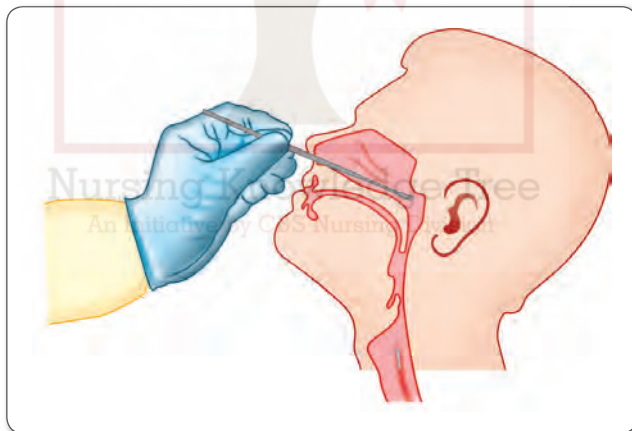
- **Most common symptoms**
 - Fever
 - Dry cough
 - Tiredness
- **Less common symptoms**
 - Aches and pains
 - Sore throat
 - Diarrhea
 - Conjunctivitis
 - Headache
 - Loss of taste or smell
 - A rash on skin, or discoloration of fingers or toes

DIAGNOSIS

At present, polymerase chain reaction (PCR), rapid antigen test and antibody testing are the dominant ways that global healthcare systems are testing citizens for COVID-19.

Sample Collection

Instructions for collecting an Nasopharyngeal (NP) specimen:



- Tilt patient's head back 70 degrees.
- Gently and slowly insert a minitip swab with a flexible shaft (wire or plastic) through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx.
- Swab should reach depth equal to distance from nostrils to outer opening of the ear.
- Gently rub and roll the swab.
- Leave swab in place for several seconds to absorb secretions.
- Slowly remove swab while rotating it.
- Specimens can be collected from both sides using the same swab, but it is not necessary to collect specimens from both sides if the minitip is saturated with fluid from the first collection.
- If a deviated septum or blockage creates difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril.

Specimen Storage

After collection, store specimens at 2–8°C for up to 72 hours. If a delay in testing or shipping is expected, store specimens at –70°C or below.

For transporting sample: Label each specimen container with the patient's ID number (e.g., medical record number), unique CDC or state-generated nCov specimen ID (e.g., laboratory requisition number), specimen type (e.g., serum) and the date the specimen was collected.

- PCR tests are used to directly detect the presence of an antigen, rather than the presence of the body's immune response, or antibodies.
- **Rapid antigen test:** Rapid antigen tests (sometimes known as a rapid diagnostic test – RDT) detect viral proteins (known as antigens). Samples are collected from the nose and/or throat with a swab as given before.

PREVENTION

- Clean your hands often. Use soap and water, or an alcohol-based hand rub.
- Maintain a safe distance from anyone who is coughing or sneezing.
- Wear a mask when physical distancing is not possible.
- Don't touch your eyes, nose or mouth.
- Cover your nose and mouth with your bent elbow or a tissue when you cough or sneeze.
- Stay home if you feel unwell.
- If you have a fever, cough and difficulty breathing, seek medical attention.

Vaccine

There are three COVID-19 vaccines for which certain national regulatory authorities have authorized the use. None has yet received WHO authorization.

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Section IV

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IMMUNOLOGY

Section Summary

CHAPTER 13 Basics of Immunology (Including National Immunization Schedule)

CHAPTER 14 Antigen-Antibody Reaction

Chapter 13

Basics of Immunology (Including National Immunization Schedule)

CHAPTER OUTLINE

- Introduction
- Innate Immunity
 - Mechanisms of Innate Immunity
- Acquired Immunity
 - Active Immunity
 - Passive Immunity
- Antigen
 - Types of Antigen
 - Properties of Antigens
- Antibody
 - Structure of Immunoglobulin
 - Types of Immunoglobulins
- Cells of Immune System
- Organs of Immune System
 - Thymus
 - Bone Marrow
 - Lymph Nodes
 - Spleen
 - Mucosa-associated Lymphoid Tissue
- Immunoprophylaxis
 - Difference between Vaccine and Sera
 - Vaccination
- Immunization
 - Combined Immunization (Vaccination)
 - National Immunization Schedule
- Hypersensitivity and Autoimmunity
 - Hypersensitivity
 - Autoimmunity

INTRODUCTION

Our body is under threat constantly by various factors like pathogenic organisms, toxins, carcinogens etc. To protect our body from these harmful factors, the defense system of the body comes into play. This defense system is also known as immune system of the body. Immunity is defined as the resistance of the body towards the harmful effects caused by the pathogenic organisms and other toxic factors. Immunity is of two types as shown in **Figure 13.1**:

1. Innate immunity
2. Acquired immunity

INNATE IMMUNITY

Innate means *inborn or natural*. This is the type of immunity, which is present since birth and is

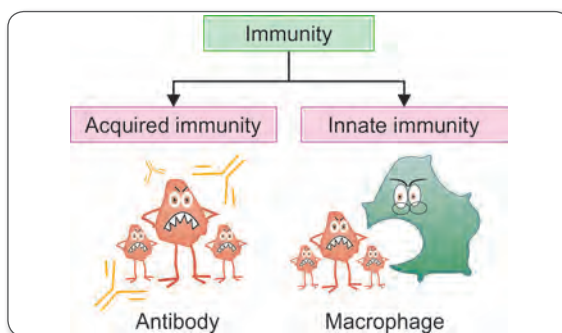


Figure 13.1: Types of immunity

possessed by an individual due to his genetic and constitutional make up. It can be nonspecific, if the resistance is against infections in general or specific, if the resistance is against a particular organism or toxin. It is considered at three levels.

1. **Species immunity:** It is the resistance shown by all members of a species. For example, Guinea pigs and humans are prone to tuberculosis, while dogs, sheep and horses are immune to it.
2. **Racial immunity:** The resistance to infection also varies amongst various races within a species. These are known to be genetic in origin. For example, black races in humans are more susceptible to tuberculosis as compared to white races.
3. **Individual immunity:** Individual immunity is the susceptibility of different individuals in a race towards an infection. For example, if a large number of people are exposed to a particular infection, some won't be affected by it, some will develop a mild infection and others will develop the infection severely.

Mechanisms of Innate Immunity

Epithelial Surfaces

- **Skin:** Skin acts as a mechanical barrier for the microorganisms to enter the body. The high concentration of salt in drying sweat, sebaceous secretions and long chain fatty acids act as bactericidal agents as well.
- **Respiratory tract:** The mucus secretion of respiratory tract traps the inhaled particles and

hair-like cilia propel the particles towards the pharynx, initiating the cough reflex. The cough reflex acts as a defense mechanism.

- **Gastrointestinal tract:** The enzymatic activity in saliva and the acidic pH of gastric juices destroy many microorganisms.
- **Conjunctiva:** Lysozyme present in tears acts as bactericidal agent. Tears also help in flushing away microorganisms and other dust particles.
- **Genitourinary tract:** Urine with its flushing action helps in eliminating bacteria from urethra. Acidic pH of vaginal secretions in females renders vagina free of many pathogens.

Complement System of the Body

Complement system plays an important role in destroying the pathogenic bacteria entering blood and tissues.

Phagocytosis by Neutrophils and Macrophages (Figure 13.2)

With the release of chemicals like histamine from damaged body cells, neutrophils and macrophages concentrate at the sites of infection. The cellular extensions, pseudopodia, surround the pathogen and engulf it forming an internal vesicle. The vesicle then fuses with the lysosome to digest the pathogen.

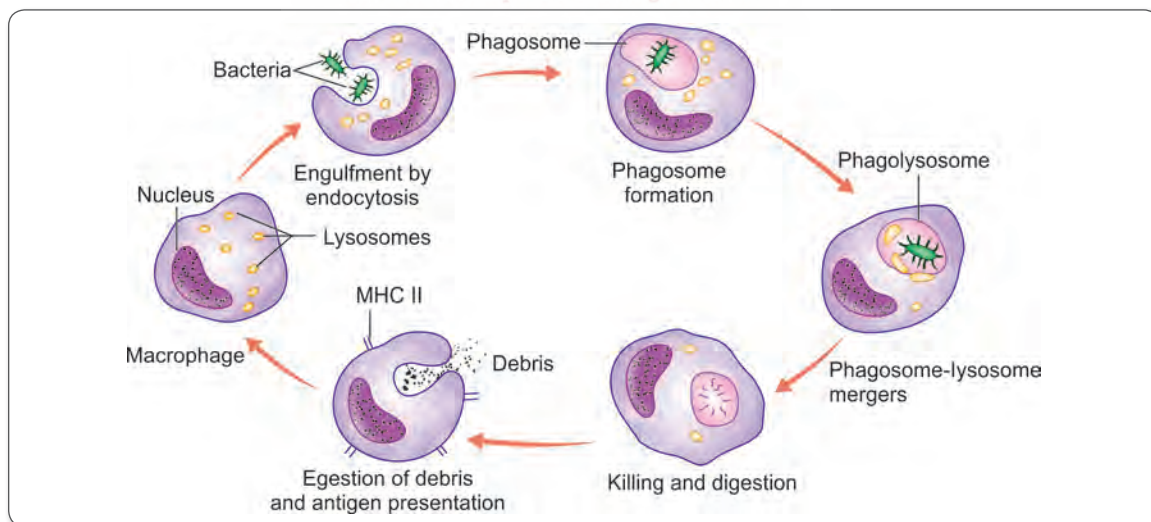


Figure 13.2: Stages of phagocytosis

Abbreviation: MHC, major histocompatibility complex

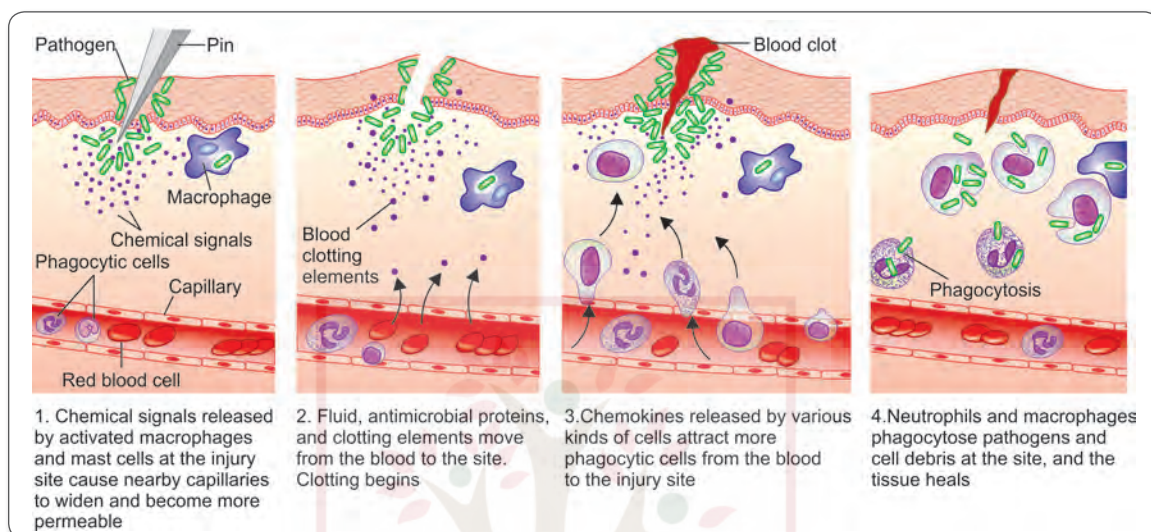


Figure 13.3: Stages of wound healing

Inflammation

With the tissue damage, mast cells release histamine that leads to local vasodilatation and increased capillary permeability. The chemotactic factors thus released, recruit wandering macrophages to the site of damage (**Figure 13.3**). Inflammation is necessary to allow immune cell to access infected tissues. The four signs of inflammation are:

1. Redness
2. Swelling
3. Heat
4. Pain

Fever

Rise in temperature or fever is a physiological response of the body that helps in accelerating the physiological processes and hence, causes destruction of microorganisms.

ACQUIRED IMMUNITY

An individual also acquires the resistance to fight against the microorganisms during his lifetime and this is called acquired immunity. It is of two types:

1. Active immunity
2. Passive immunity.

Active Immunity

It is induced by an infection or by contact with antigens. It can be either natural (produced due to clinical or subclinical infection) or artificial (induced by vaccination).

- **Natural active immunity:** It is resistance developed from clinical or subclinical infections.
 - **Long-lasting immunity:** Diphtheria, measles, mumps, whooping cough
 - **Short-time immunity:** Influenza and common cold.
- **Artificial active immunity:** This is the resistance that is induced by vaccines in the body. Vaccines are prepared from live, attenuated or killed microorganisms, or their antigens and toxins.
 - **Bacterial vaccines:**
 - ◆ Live: Ty21a for typhoid, Bacillus Calmette Guérin (BCG) for tuberculosis
 - ◆ Killed: Cholera, pertussis, TAB for enteric fever
 - **Bacterial products:** Tetanus, diphtheria toxoid
 - **Viral vaccines:**
 - ◆ **Live:** Sabin vaccine for polio, MMR for mumps, measles and rubella
 - ◆ **Killed:** Salk vaccine for polio, hepatitis B vaccine.

TABLE 13.1: Differences between Active and Passive Immunity

Active immunity	Passive immunity
The immune system of the body actively takes part in response to the exposure of antigenic material	The immune system does not actively participate and this immunity is received passively by the host
It is induced by infection or immunogens	Administration of ready-made antibodies is done
It is long-lasting and more effective	It is short-lived and less efficacious
Antibodies take some time in generation. Hence, this immunity is effective only after a lag period	Immunity comes in effect immediately as ready-made antibodies are administered
Immunological memory is present	Immunological memory is absent
It is not effective and applicable in immunodeficient persons	It is effective and applicable in immunodeficient persons

Also Know

In killed vaccines, the organisms are killed by heat, formalin, alcohol and phenol. Toxoids are prepared from inactivating the bacterial exotoxins by formalin or alum. Toxoids are immunogenic and are not toxigenic.

Mechanisms of Active Immunity

Active immunity stimulates both humoral and cell-mediated immunity.

- **Humoral immunity:** Humoral immunity or antibody-mediated immunity depends on the synthesis of antibodies by plasma cells. The specific antibodies, thus produced, combine with specific antigens and modify their activity.
- **Cell-mediated immunity:** The cell-mediated immunity (by sensitized T-lymphocytes) is important in resistance to chronic bacterial infections.

Passive Immunity

In passive immunity, the immune system of the individual plays no active role. Readymade antibodies are transferred into the individual. It is short-lived and is useful when immunity is required immediately. It is of two types:

1. **Natural:** It includes transfer of maternal antibodies (IgG) transplacentally to the fetus and to the infant through milk. It provides immediate protection to the infant/fetus and protects them till their own immune system matures.

2. **Artificial:** Parenteral administration of antibodies is done in this type of passive immunity. The agents used are hyperimmune sera of animal or human origin, convalescent sera and pooled human gamma globulin.

The difference between active and passive immunity are summarized in **Table 13.1**.

Also Know

Passive immunity is also induced for suppression of active immunity. For example, in Rh negative mothers with Rh positive babies, Rh immunoglobulin is induced during delivery to prevent immune response to Rh factor.

Terms to Learn

- **Combined immunization:** A combination of active and passive immunization is employed simultaneously in this case. Passive immunity provides protection till the active immunity becomes effective.
- **Herd immunity:** The overall resistance in a community is called herd immunity. If herd immunity is low, outbreaks of epidemics increase on introduction of a suitable pathogen.

ANTIGEN

An antigen is a substance, which stimulates the production of a specific antibody, after it enters the body.

Types of Antigen

Antigens are of two types:

1. **Complete antigen:** These can induce antibody formation by themselves and can react specifically with these antibodies.
2. **Haptens:** These cannot induce antibody formation, but after covalently linking to a carrier protein, it is capable of inducing antibody formation.

Also Know

The smallest unit of antigenicity is called *epitope* or *antigenic determinant*. They have a specific chemical structure, electrical charge and induce a specific antibody formation, which reacts at that site.

Properties of Antigens

- **Foreignness:** In order to induce an immune response, an antigen should be foreign to the body.
- **Size:** Larger the molecular size of the antigen, higher is the antigenicity.
- **Chemical nature:** Out of the 4 major biomolecules, proteins are more effective antigens.
- **Species specificity:** Tissues of all individuals in a species contain species-specific antigens. It plays an important role in evolutionary relationship.
- **Isospecificity:** Isoantigens are found only in some members of a species. This helps in grouping of the species according to the presence of an isoantigen. Blood grouping is one example depending on human erythrocyte antigens.
- **Autospecificity:** Auto means *self*. Autospecific antigens are generally nonantigenic.
- **Organ specificity:** The organ specific antigens are confined to a particular organ. Organs like lens protein, brain and kidney of one species share specificity with that of another species.
- **Heterogenic specificity:** Some closely related antigens are found in different biological species like bacteria, plants and animals. These are called heterogenic or heterophile antigens.

Also Know

Heterophile antigens are used for serological diagnosis of several diseases.

ANTIBODY

Antibody or immunoglobulin is a substance produced in the body in response to an antigen and reacts with it specifically.

Structure of Immunoglobulin

Immunoglobulins are glycoprotein in nature and each molecule consists of two identical heavy (H) chains that are longer in size and two identical light (L) chains that are shorter in size. The 2 H chains are joined by 1–5 disulfide bonds and the L and H chains are joined by disulfide bonds (**Figure 13.4**). The H chains are structurally and antigenically different for each class of immunoglobulins. There are five classes of immunoglobulins namely, IgG, IgM, IgA, IgD and IgE.

Also Know

The five classes of immunoglobulins, IgG, IgM, IgA, IgD and IgE are designated depending on presence of heavy chain gamma (γ), mu (μ), alpha (α), delta (δ) and epsilon (ϵ), respectively.

Light chains are similar in all classes. They occur in two forms: kappa (κ) and lambda (λ). An immunoglobulin has either 2 kappa or 2 lambda chains, but never both.

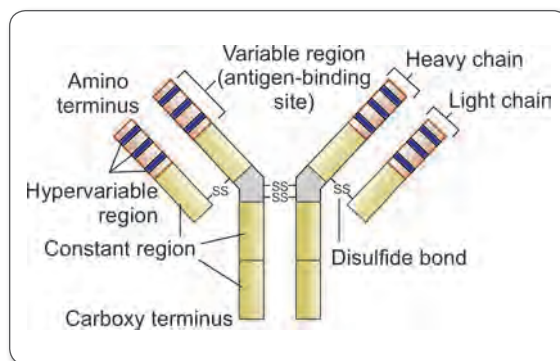


Figure 13.4: Structure of immunoglobulin

TABLE 13.2: Properties of Immunoglobulins

Property	IgG	IgA	IgM	IgD	IgE
Molecular weight	150,000	160,000	900,000	180,000	190,000
Heavy chain	γ	α	μ	δ	ϵ
Serum conc. (mg/mL)	12	2	1.2	0.03	0.0004
Half-life (days)	23	6–8	5	3	2–3
Placental transport	Present	Absent	Absent	Absent	Absent
Present in mother's milk	Present	Present	Absent	Absent	Absent
Heat stability	Yes	Yes	Yes	Yes	No

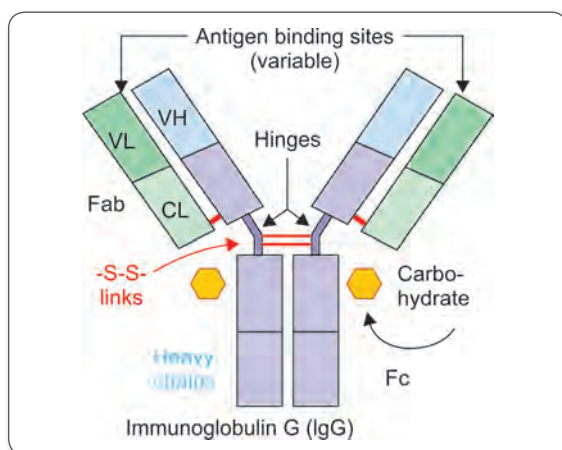
The site for combining with antigen is composed of both H and L chains. This part has a constant region called carboxy terminus and other variable terminal is called amino terminus.

Types of Immunoglobulins

Few important properties of various immunoglobulins are summarized in **Table 13.2**.

IgG

It is the major serum immunoglobulin and constitutes about 80% of all immunoglobulins (**Figure 13.5**). It is the only antibody that passes through the placenta. It is involved in complement activation and phagocytosis and protects against the microorganisms, which are active in blood and tissues.

**Figure 13.5:** Structure of IgG

IgA

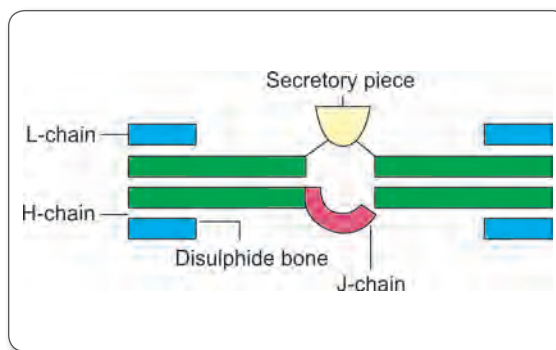
It is the second most abundant class of immunoglobulins and constitutes about 10–13% of total serum immunoglobulins. It is a principal immunoglobulin present in milk, saliva, tears, sweat, nasal fluids, and colostrum and in secretions of respiratory, intestinal and genital system. Initially it is a monomer and it attains a secretory component later. It becomes dimer linked by J-chains and is known as secretory IgA (**Figure 13.6**).

IgM

IgM is the largest antibody and the first immunoglobulin that is produced on exposure. It is the third most abundant immunoglobulin. It is responsible for complement activation and forms ABO antibodies. It is a pentamer (**Figure 13.7**).

Also Know

The molecular wt. of IgM is 900,000–1,000,000 and hence, it is called a millionaire molecule.

**Figure 13.6:** Structure of secretory IgA molecule

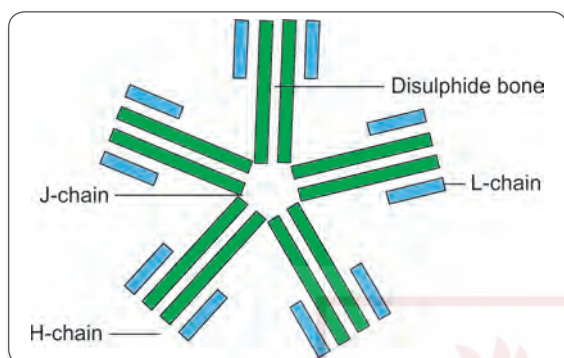


Figure 13.7: Structure of IgM molecule

IgD

It is a monomer and is similar to IgG in structure. It is short-lived and plays an important role in secondary immune response. It occurs in combination with IgM on surface of B lymphocytes and is involved in antigen recognition by B cells.

IgE

It is extravascular in distribution and is a monomer. It is responsible in the release of histamine from basophil and mast cells and hence, it is involved in anaphylactic type of hypersensitivity. It is produced by respiratory and intestinal tract linings. It is increased in atopic allergy and parasitic infestations.

CELLS OF IMMUNE SYSTEM

The various cells along with their functions have been summarized in **Table 13.3**.

Also Know

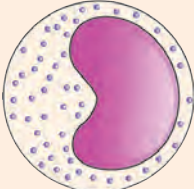
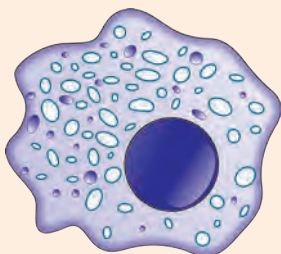
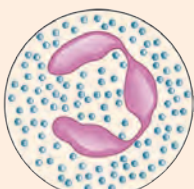
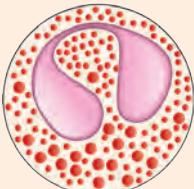
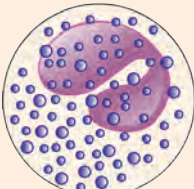
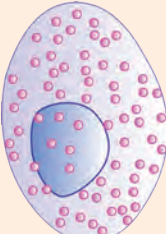
Specific tissue macrophages are as follows:

- **Liver:** Kupffer cells
- **Lung:** Alveolar macrophages
- **Connective tissue:** Histiocytes
- **Bone:** Osteoclasts
- **Brain:** Microglial cells.


TABLE 13.3: Characteristics of Cells of Immune System

Cell type	Characteristics	Image
T-lymphocytes	Mature in thymus; possess T-cell receptor; responsible for cell-mediated immunity; three types of T lymphocytes: 1. T-helper (T_H) cells: Recognize and interact with antigen 2. T-suppressor (T_S) cells: Suppress cell-mediated and humoral immunity 3. Cytotoxic T (T_C) cells: Activated under influence of cytokines	
B-lymphocytes	Mature in bone marrow; contain unique IgM receptor; responsible for humoral immunity; after activation, divides into two types: 1. Memory B cells: Express membrane bound antibody 2. Effector B cells or plasma cells: Produce antibodies	
Natural killer cells	Kills tumor cells and virus-infected cells; action is independent of antibody; activity does not require sensitization by prior antigenic contact	

Contd...

Cell type	Characteristics	Image
Monocytes	Stored in spleen; differentiates into macrophages and dendritic cells in response to inflammation	
Macrophages	Phagocytic cell that consumes foreign pathogens and cancer cells; stimulates response of other immune cells	
Neutrophils	First to respond at the site of infection or trauma; most abundant of all leukocytes; causes release of toxins that kill or inhibit bacteria or fungi	
Eosinophils	Less phagocytic than neutrophils; found in large numbers in allergic inflammation and parasitic infections	
Basophils	Present in blood and tissues; responsible for defense against parasites; releases histamines that cause inflammation and may be responsible for allergic reactions	
Mast cells	Present in connective tissues and mucous membranes; dilates blood vessels and induces inflammation through release of histamine and heparin; involved in wound healing and defense against pathogens	

Contd...

Cell type	Characteristics	Image
Dendritic cells	Present in epithelial tissues including skin, lung and gastrointestinal tract; presents antigen on its surface, thereby triggering adaptive immunity	

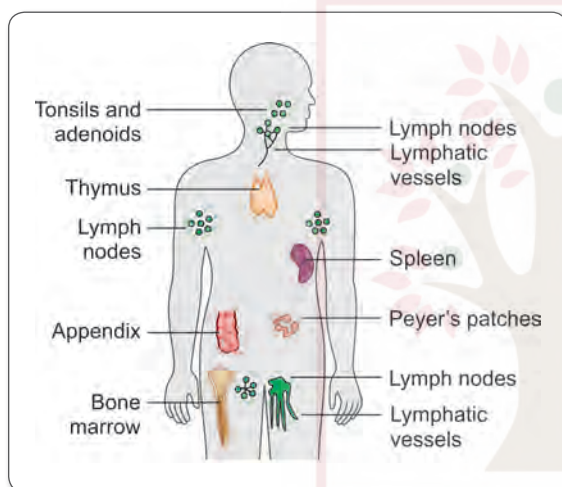


Figure 13.8: Organs of immune system

ORGANS OF IMMUNE SYSTEM

The important organs of the immune system (Figure 13.8) have been described below.

Thymus

- Greyish, flat, bilobed lymphoid organ present in the upper part of chest cavity
- Primary function is to produce T lymphocytes
- Grows in size till puberty and later regresses in size
- Precursors of T lymphocytes are present in cortex; they pass to medulla after maturation. From medulla, they migrate to secondary lymphoid organs.

Bone Marrow

- Site of origin of B Lymphocytes
- Equivalent to bursa of Fabricius in birds

Lymph Nodes

- Small bean-shaped clusters present along the course of lymphatic vessels
- Act as filters of lymph
- Lymphocytes, macrophages and dendritic cells are arranged in follicles in the cortex area.

Spleen

- Largest lymphoid organ
- Traps blood-borne antigens
- Red pulp is rich in red blood cells and macrophages and white pulp contains primary and secondary lymphoid follicles having B-lymphocytes.

Mucosa-associated Lymphoid Tissue

- Includes Peyer's patches of small intestine, tonsils, appendix, salivary glands, lacrimal glands and follicles in respiratory tract
- Present diffusely in submucosa and mucosa
- Contains both T and B lymphocytes.

Also Know

- Polio vaccines must be stored at minus 20°C.
- Typhoid, Diphtheria and tetanus toxoids (DT), DPT, BCG and tetanus vaccines should never be freeze dried.

IMMUNOPROPHYLAXIS

It is the prevention of disease by the production of active or passive immunity. Vaccination is used for this purpose by using vaccines, antisera or immune serum globulin (human).

Difference between Vaccine and Sera

Vaccine therapy for prevention or cure of infection has for its object the production of an active

immunity to the specific bacteria concerned, while serum therapy produces a passive immunity only.

Know it.....

Cold Chain

Cold chain is a system of storing and transporting vaccines at a low temperature, ideally recommended for the vaccines. Maintenance of cold storage is important as vaccines may lose their potency if not properly stored at right temperature. All vaccines must be stored between 2° and 8°C.

Vaccination

- Immunity against pathogens (viruses and bacteria) is obtained by using: live attenuated, killed or altered antigens that stimulate the body to produce antibodies.
- Vaccines work with the immune system's ability to recognize and destroy foreign proteins (antigens).
- Vaccination prevents and control such diseases as cholera, rabies, poliomyelitis, diphtheria, tetanus, measles, and typhoid fever.

Vaccines

- Vaccines can be:
 - Prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen.
 - Therapeutic (e.g. vaccines against cancer that are still under investigation).

Types of Vaccines

- **Killed vaccines:** Virulent bacteria or viruses used to prepare these vaccines may be killed by heat (60°C) or by chemicals (formalin, phenol or merthiolate), for examples:
 - TAB vaccine against enteric fever (heat)
 - Salk vaccine against poliomyelitis (formalin)
 - Semple's vaccine against rabies (phenol)
 - Pertussis vaccine against whooping cough (merthiolate)

Characteristics of killed vaccine:

- ◆ Do not stimulate local immunity
- ◆ Short lasting

- ◆ Do not stimulate cytotoxic T cell response in contrast to live attenuated vaccines
- ◆ Safe can be given to pregnant woman and immunocompromised host
- ◆ It is heat stable

- **Live attenuated vaccines:** Living microorganism loses its virulence so does not produce disease but produces immunity. It stimulates both humoral and cell mediated immunity, local and systemic. It is not given to pregnant women and immunocompromised hosts (may cause diseases). It is heat labile.

It is prepared by:

- Repeated subculture in unsuitable condition (chemical or media), e.g. BCG vaccine against T.B. and 17 D vaccine against yellow fever.
- Growing at high temperature (Above optimum temp), e.g. Pasteur anthrax vaccine
- Selection of mutant strains of low virulence, e.g., Sabin vaccine against poliomyelitis.
- **Toxoids:** It is prepared by detoxifying bacterial toxins. Bacterial exotoxins are treated by formalin to destroy toxicity and retain antigenicity, e.g., diphtheria and tetanus toxoid.
- **Microbial products vaccines** are prepared from bacterial products or viral components, e.g., Capsular polysaccharide vaccines are:
 - Poor immunogen in children below 2 years age, e.g., *H. influenzae* do not respond to T cell independent antigens in spite of its generation of IgM
 - Produce anticapsular opsonizing antibodies, for examples meningococci, pneumococci and *H. influenzae*
 - Cellular purified proteins of pertussis
 - Purified surface Ag of hepatitis B virus
 - Influenza viruses

Prepared by recombinant DNA technology for improvement vaccines, e.g.

- ◆ Subunit vaccines in which microbial polypeptides are isolated from the infective material hepatitis B and influenza viruses
- ◆ Recombinant DNA-derived antigen vaccines in which Ag are synthesizing

- by inserting the coding genes into *E. coli* or yeast cell as HBV vaccines
- ♦ Recombinant DNA avirulent vector vaccines in which the genes coding for the Ag is inserted into genome of an avirulent vector such as BCG vaccine
- ♦ **Synthetic peptide vaccines:** Synthesis of short peptides that correspond to antigenic determinants on a viral or bacterial proteins, e.g cholera toxins and poliovirus to produce Ab response.
- **Bacterial vaccines:**
 - **Live:** Ty21a for typhoid, BCG for tuberculosis
 - **Killed:** Cholera, pertussis, TAB for enteric fever
- **Bacterial products:** Tetanus, diphtheria toxoid
- **Viral vaccines:**
 - Live: Sabin vaccine for polio, MMR for mumps, measles and rubella
 - Killed: Salk vaccine for polio, hepatitis B vaccine
- **Combined vaccines:** Diphtheria, pertussis (DPT), MMR

IMMUNIZATION

Immunization is the process by which resistance to an infection is either induced or enhanced. The primary purpose of immunization is to prevent, control and eradicate various infections. Immunization can be achieved by vaccines. Vaccines are prepared from live, attenuated or killed microorganisms, or their antigens and toxins and are used to induce active immunity to an infectious agent. Examples of commonly used vaccines are as follows.


Combined Immunization (Vaccination)

Immunization against diseases is recommended in combination (for young children) as:

- Diphtheria, tetanus (lockjaw), and pertussis (whooping cough), given together (DTP).
- Measles, mumps, and rubella, give together as MMR
- Haemophilus influenzae b (Hib) with DTP
- Influenzae b (Hib) with inactivated poliomyelitis vaccine (IPV)
- Influenza and *Neisseria meningitides* (Meningococcal meningitis).

National Immunization Schedule (Table 13.4)

TABLE 13.4: National Immunization Schedule for Infants, Children and Pregnant Women

For Pregnant Women						
Vaccine	When to give	Dose	Diluent	Route	Site	
TT-1	Early in pregnancy	0.5 mL	No	Intramuscular	Upper arm	
TT-2	4 weeks after TT-1	0.5 mL	No	Intramuscular	Upper arm	
TT-Booster	If received TT doses in a pregnancy within last 3 years	0.5 mL	No	Intramuscular	Upper arm	

For Infants						
Vaccine	When to give	Max. Age	Dose	Diluent	Route	Site
BCG	At birth as early as possible	Till 1 year of age	0.1 mL (0.05 mL until 1 month age)	Sodium chloride	Intradermal	Left upper arm
Hepatitis B birth dose	At birth as early as possible	Within 24 hours	0.5 mL	NO	Intramuscular	Anterolateral side of mid-thigh LEFT

Contd...

OPV-0	At birth as early as possible	Within the first 15 days	2 drops	NO	Oral	
OPV 1, 2 & 3	At 6, 10 and 14 weeks	Till 5 years of age	2 drops	NO	Oral	
Rota virus vaccine	At 6, 10 and 14 weeks	Till 1 year of age	5 drops	NO	Oral	
IPV (inactivated polio vaccine)	At 6 and 14 weeks	Up to 1 year of age	0.1 mL	NO	Intradermal	Right upper arm
Pentavalent 1, 2 and 3	At 6, 10 and 14 weeks	Till 1 year of age	0.5 mL	NO	Intramuscular	Anterolateral side of mid-thigh LEFT
Measles 1st dose	9–12 completed months	Given till 5 years of age	0.5 mL	Sterile water	Subcutaneous	Right upper arm
Japanese Encephalitis 1st dose	9–12 completed months	Till 15 years	0.5 mL	Phosphate buffer	Subcutaneous	Left upper arm
Vitamin A (1st dose)	At 9 completed months with measles	Till 5 years of age	1 mL (1 lakh IU)	NO	Oral	
For Children						
DTP Booster –1	16–24 months	7 years	0.5 mL	NO	Intramuscular	Anterolateral side of mid-thigh LEFT
Measles 2nd dose	16–24 months	Till 5 years of age	0.5 mL	Sterile water	Subcutaneous	Right upper arm
OPV Booster	16–24 months	Till 5 years of age	2 drops	NO	Oral	
Japanese Encephalitis 2nd dose	16–24 months		0.5 mL	Phosphate buffer	Subcutaneous	Left upper arm
Vitamin A (2nd to 9th dose)	16 months. Then, 1 dose every 6 months	Till 5 years of age	2 mL (2 lakh IU)	NO	Oral	
DPT Booster –2	5–6 years	7 years	0.5 mL	NO	Intramuscular	Upper arm (Left)
TT	10 years and 16 years		0.5 mL	NO	Intramuscular	Upper arm

Note:

- Give TT-2 or Booster doses before 36 weeks of pregnancy. However, give these even if more than 36 weeks have passed. Give TT to a woman in labour, if she has not previously received TT.
- JE Vaccine, in select endemic districts after the campaign.
- The 2nd to 9th doses of Vitamin A can be administered to children 1-5 years old during biannual rounds, in collaboration with ICDS.

HYPERSENSITIVITY AND AUTOIMMUNITY

Hypersensitivity

Although immune responses are meant to protect our body, sometimes they might cause excessive reactions which leads to damage of the tissues, development of some disease or even death in a sensitized host. Such a reaction is called hypersensitivity reaction. They are either “immediate” i.e. reaction appears quickly and “delayed” i.e. reactions appear slowly in 24–72 hours. Coomb and Gel gave the classification of hypersensitivity reactions in 1963 and divided it in four major types that are summarized in **Table 13.5**.

Autoimmunity

Our immune system has been developed in a way that it recognizes difference between the cells of the body and foreign substances. Autoimmunity can be defined as a condition in which our immune system reacts against body’s own cells by making antibodies. This leads to functional and structural damage to the tissues. The diseases produced as a result of such a reaction are called autoimmune diseases. Common examples of autoimmune diseases are, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis, type 1 diabetes mellitus, Grave’s disease, myasthenia gravis, etc.

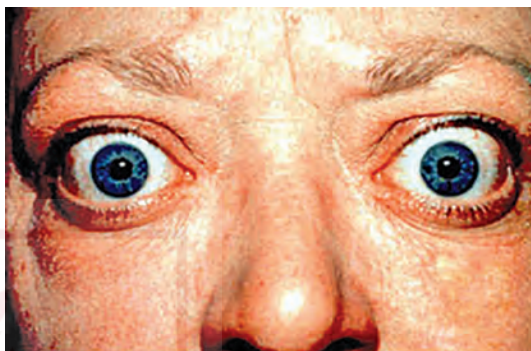
TABLE 13.5: Four Major Types of Hypersensitivity Reactions (Coomb and Gel)

Type	Name	Mechanism	Disease examples
Type I	Immediate hypersensitivity	IgE-mediated degranulation of mast cells following antigen binding and cross-linking of IgE	Allergic asthma, allergic rhinitis, anaphylaxis
Type II	Antibody-mediated hypersensitivity	IgM/IgG antibody: antigen interactions on target cell surfaces	Drug-induced thrombocytopenia, myasthenia gravis, Graves disease, hemolytic anemia of newborn
Type III	Immune complex-mediated hypersensitivity	Immune complex formation and deposition in tissues leading to local or systemic inflammatory reactions	Rheumatoid arthritis, systemic lupus erythematosus (SLE), Good Pasture’s syndrome, arthus reaction, serum sickness
Type IV	Delayed-type hypersensitivity	Sensitized T_H1 cells activated to release cytokines upon binding to antigen, resulting in macrophage and cytotoxic T cell accumulation	Contact dermatitis, chronic transplant rejection

Color Plates of Autoimmune Diseases



Color plate 1: SLE



Color plate 2: Graves



Color plate 3: Arthritis



Color plate 4: Myasthenia gravis



Assess Yourself

LONG ANSWER QUESTIONS

1. Define immunity.
2. Explain the types of immunity.

SHORT NOTES

1. Hypersensitivity
2. Types of immunity

MULTIPLE CHOICE QUESTIONS

1. Which antibody crosses placenta?
 - a. IgA
 - b. IgG
 - c. IgE
 - d. IgM
2. Type I hypersensitivity is mediated by which of the following immunoglobulins?
 - a. IgA
 - b. IgG
 - c. IgM
 - d. IgE
3. Zero dose of OPV is given:
 - a. At one month
 - b. At birth
 - c. When child is having diarrhea
 - d. When child is having polio
4. Which of the following is live attenuated vaccine?
 - a. BCG vaccine
 - b. Rabies vaccine
 - c. Diphtheria toxoid
 - d. Tetanus toxoid
5. Which antibody is responsible for allergic responses?
 - a. IgG
 - b. IgA
 - c. IgD
 - d. IgE
6. Anaphylaxis is:
 - a. Type I hypersensitivity
 - b. Type II hypersensitivity
 - c. Type III hypersensitivity
 - d. Type IV hypersensitivity
7. BCG is a:
 - a. Live attenuated vaccine
 - b. Killed vaccine
 - c. Toxoid
 - d. Immunoglobulin

ANSWERS TO MCQs

1. b 2. d 3. b 4. a 5. d 6. a 7. a

Chapter

14

Antigen-Antibody Reaction

CHAPTER OUTLINE

- Introduction
- Serological Tests
 - Principles of Serological Tests
- Precipitation Reactions
 - Ring Test
 - Flocculation Test
- Agglutination Reactions
 - Slide Agglutination Test
 - Tube Agglutination Test
- Complement Fixation Test
- Immunofluorescence
- Enzyme Linked Immunosorbent Assay (ELISA)
- Uses of Serological Tests

INTRODUCTION

Antigens combine with their specific antibodies in a specific and observable manner. The reaction is reversible and complete molecules of antigen and antibody react with each other. While these reactions provide protection to the body against various diseases, they can be used to diagnose infections and detection of antigens or antibodies in the laboratory.

SEROLOGICAL TESTS

Principles of Serological Tests

- Serological diagnosis is usually based on either the demonstration of the presence of specific IgM antibodies or a significant increase in the levels of specific IgG antibodies between two consecutive samples taken 1–4 weeks apart.
- The antigen for the test can be either viable or inactivated virus or some of its components prepared by virological or molecular methods.
- Isotype-specific markers or physical separation are used to demonstrate the isotype of the reacting antibody.

Serological tests based on antigen-antibody reactions are as follows.

PRECIPITATION REACTIONS

When a soluble antigen reacts with an antibody in the presence of electrolytes like sodium chloride (NaCl) at a specific temperature and pH, they form an antigen-antibody complex (in the form of precipitate) that settles down at the bottom of the test tube. This reaction is called precipitation reactions. When this complex does not settle down but remains suspended in the test tube, it is called flocculation.

Ring Test

In this technique, antiserum containing antibodies is put in a narrow test tube and antigen solution is layered over it. A precipitate ring appears where the two liquids meet. It is used for C-reactive protein test and streptococcal grouping.

Flocculation Test

It can be done either on a slide or in a test tube.

- **Slide test:** A drop of inactivated serum of patient is put on a slide and a drop of antigen solution is added over it and they are mixed by shaking. It leads to appearance of suspended complexes called floccules. Venereal Disease Research Laboratory (VDRL) test is an example of slide test and is used to detect antibodies against syphilis.
- **Tube test:** The Kahn test is an example of tube test and was done previously for diagnosis of syphilis.

AGGLUTINATION REACTIONS

In this type of antigen-antibody reaction, a particulate antigen combines with its antibody in the presence of electrolytes like sodium chloride (NaCl) and optimal temperature and pH. There is visible clumping of particles.

Slide Agglutination Test

In this, a drop of antiserum is added to the uniform suspension of antigen and saline on the slide. If immediate clumping is seen, the test is positive. This is generally used for blood group determination and cross matching.

Tube Agglutination Test

In this, the serum is serially diluted by increasing the dilution by two times in a series of test tubes. Particulate antigen is added in equal volume to all the tubes. The highest dilution of serum at which agglutination occurs is the antibody titer. This technique is used in routine in Widal test for diagnosis of typhoid.

COMPLEMENT FIXATION TEST

Complement is absorbed during combinations of antigens and antibodies while taking part in various immunological reactions. This is based on the principle that the antigen-antibody complexes have the ability to 'fix' complement. It is a very sensitive test and can detect even the smallest amounts of

antigen and antibody. Diseases like gonorrhea, syphilis, typhus fever, Kala-azar, etc. are diagnosed by this technique.

IMMUNOFLUORESCENCE

Fluorescent dyes absorb invisible ultraviolet light and emit visible green light, which has a longer wavelength. Therefore, if microorganisms or any cell is stained with a fluorescent dye and examined under the microscope with ultraviolet light, they are seen as bright objects against a dark background. This principle is used in fluorescence microscopy. Coons and his colleagues showed that fluorescent dyes can be conjugated to antibodies, leading to their ready detection when attached to an antigen associated with the cell. This method is used for diagnosing rabies virus antigens, syphilis, and detection of autoantibodies.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

It is an enzyme-based test that can detect either antigens or antibodies in the serum sample of patient. The principle for this method is same as that of immunofluorescence. The only difference is that instead of dyes, enzyme is being used. The enzyme acts on substrate to produce a color in a positive test. This is the most commonly used serological test that is being used to diagnose diseases like tuberculosis, hepatitis B and C, HIV infection, rubella and herpes simplex virus.

USES OF SEROLOGICAL TESTS

For the diagnosis of certain bacterial, parasitic, and viral diseases, including measles, polio, influenza, yellow fever, Rocky mountain spotted fever and infectious mononucleosis.

These are also useful in the detection of autoantibodies (harmful antibodies that attack components of the body) that are involved in autoimmune diseases, such as arthritis.

As a practical mass-screening tool, serological testing has proved valuable in the detection of diseases such as syphilis, AIDS and COVID.



Assess Yourself

LONG ANSWER QUESTION

1. Discuss uses of serological tests.

MULTIPLE CHOICE QUESTIONS

1. Ring test is used to detect:
 - a. Streptococcus
 - b. Retrovirus
 - c. Pneumococcus
 - d. Both A and C
2. Kahn test is:
 - a. Slide test
 - b. Ring test
 - c. Tube test
 - d. Complement fixation test
3. Immunofluorescence is used for diagnosing all except:
 - a. Rabies virus
 - b. Syphilis
 - c. Detection of autoantibodies
 - d. Streptococcal infection
4. Complement fixation test is used to diagnose all except:
 - a. Syphilis
 - b. Gonorrhea
 - c. Kala-azar
 - d. Rabies virus
5. Schick test is done for the diagnosis of:
 - a. Rubella
 - b. Measles
 - c. Diphtheria
 - d. Mumps

ANSWERS TO MCQS

1. a
2. c
3. d
4. d
5. c

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Chapter 16

Biomedical Waste Management

CHAPTER OUTLINE

- Introduction
- Biomedical Waste
 - Different Types of Biomedical Waste according to WHO
- Biomedical Waste Management
 - Collection of Biomedical Waste
 - Transportation
 - Treatment of Hospital Waste
- Safety Measures
 - Universal Precautions
 - Personal Protection

INTRODUCTION

All human activities produce waste. We all know that such waste may be dangerous and needs safe disposal. Industrial waste, sewage and agricultural waste pollute water, soil and air. It can also be dangerous to human beings and environment. Similarly, hospitals and other health care facilities generate lots of waste which can transmit infections, particularly HIV, Hepatitis B and C and Tetanus, to the people who handle it or come in contact with it.

BIOMEDICAL WASTE

Biomedical waste means “any solid and/or liquid waste including its container and any intermediate product, which is generated during the diagnosis, treatment or immunization of human beings or animals”. Biomedical waste poses hazard due to two principal reasons—the first is infectivity and the other, toxicity.

Biomedical waste consists of:

- Human anatomical waste like tissues, organs and body parts

- Animal wastes generated during research from veterinary hospitals
- Microbiology and biotechnology wastes
- Waste sharp instruments like hypodermic needles, syringes, scalpels and broken glass
- Discarded medicines and cytotoxic drugs
- Soiled waste such as dressing, bandages, plaster casts, material contaminated with blood, tubes and catheters
- Liquid waste from any of the infected areas
- Incineration ash and other chemical wastes

Different Types of Biomedical Wastes According to WHO

The World Health Organisation (WHO) has classified medical wastes according to their weight, density and constituents into different categories. These are:

- **Infectious:** Material-containing pathogens in sufficient concentrations or quantities that, if exposed, can cause diseases. This includes waste from surgery and autopsies on patients with infectious diseases, sharps, disposable needles, syringes, saws, blades, broken glasses, nails or any other item that could cause a cut.

- **Pathological:** Tissues, organs, body parts, human flesh, fetuses, blood and body fluids, drugs and chemicals that are returned from wards, spilled, outdated, contaminated, or are no longer required
- **Radioactive:** Solids, liquids and gaseous waste contaminated with radioactive substances used in diagnosis and treatment of diseases like toxic goiter
- **Others:** Waste from the offices, kitchens, rooms, including bed linen, utensils, paper, etc.

BIOMEDICAL WASTE MANAGEMENT

The biomedical waste management requires its categorization as a first step. The biomedical waste rules classify the biomedical waste into 9 categories. (Table 16.1)

The key to minimization and effective management of biomedical waste is segregation

(separation) and identification of the waste. The most appropriate way of identifying the categories of biomedical waste is by sorting the waste into color-coded plastic bags or containers. Biomedical waste should be segregated into containers/bags at the point of generation (Figure 16.1 and Table 16.1).

Collection of Biomedical Waste

Collection of biomedical waste should be done as per biomedical waste (management and handling) rules. At ordinary room temperature, the collected waste should not be stored for >24 hours.

Transportation

Within hospital, waste routes must be designated to avoid the passage of waste through patient care areas. Separate time should be marked for transportation of biomedical waste to reduce chances of its mixing with general waste. Desiccated wheeled containers, trolleys or carts should be used to transport the waste/plastic bags to the site of storage/treatment.

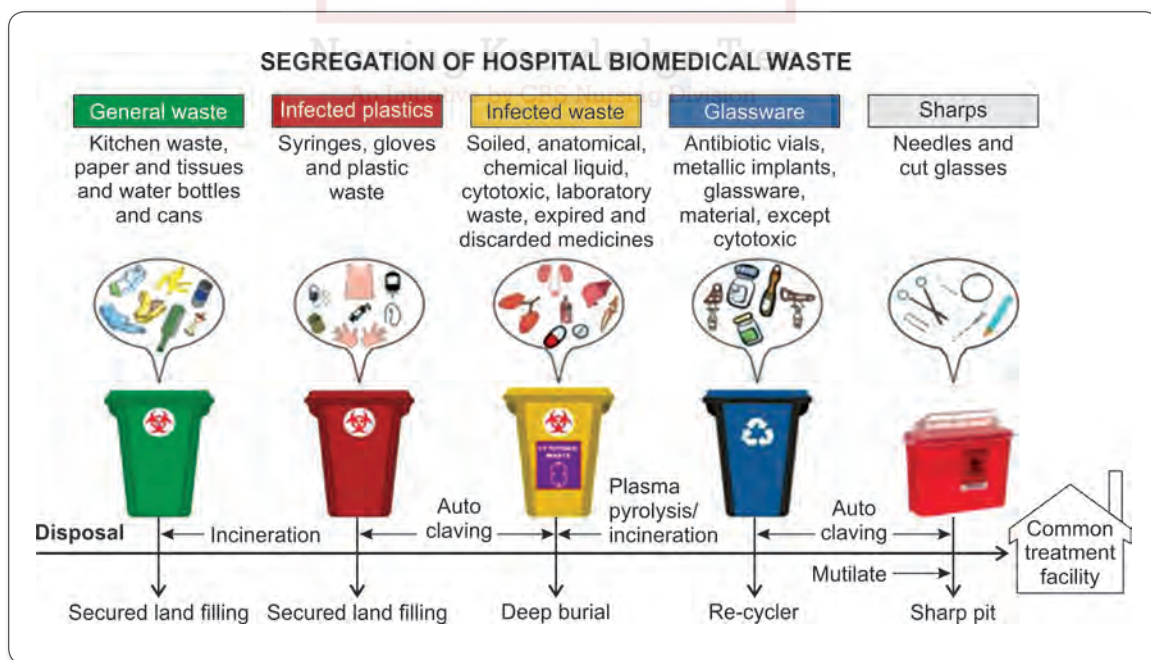


Figure 16.1: Segregation of biomedical waste

TABLE 16.1: Categories of Biomedical Waste Schedule – I

Category	Description of waste category	Treatment and disposal
Category 1	Human anatomical waste: Human tissues, organs, body parts.	Incineration/deep burial
Category 2	Animal waste: Animal tissues, organs, body parts, carcasses, bleeding parts, fluid, blood and experimental animals used in research, waste generated by veterinary hospitals colleges, discharge from hospitals, animal houses.	Incineration/deep burial
Category 3	Microbiology and biotechnology waste: Wastes from laboratory cultures, stocks of specimens of live microorganism live or attenuated vaccines, human and animals cell culture used in research, infectious agents from research and industrial laboratories, wastes from production of biological, toxins, dishes and devices used for transfer of cultures.	Autoclaving/microwaving/incineration
Category 4	Waste sharps: Needles, syringes, scalpels, blades, glass, etc. that may cause puncture and cuts. This includes both used and unused sharps.	Disinfecting (chemical treatment/autoclaving/microwaving and mutilation/shredding)
Category 5	Discarded medicine and cytotoxic drugs: Wastes comprising of outdated, contaminated and discarded medicines.	Incineration/destruction and drugs disposal in secured landfills
Category 6	Soiled waste: Items contaminated with bloods fluids including cotton, dressings, soiled plaster casts, linens, bedding, other materials contaminated with blood.	Incineration/autoclaving/microwaving
Category 7	Solid waste: Waste generated from disposable items other than the waste sharps such as tubing, catheters, intravenous sets, etc.	Disinfecting/autoclaving/microwaving and mutilation/shredding
Category 8	Liquid waste: Waste generated from laboratory and washing, cleaning, housekeeping and disinfecting activities.	Disinfecting/discharge into drains
Category 9	Incineration ash: Ash from incineration of any biomedical waste.	Disposal of secured landfill

Treatment of Hospital Waste

General Waste

The 85% of the waste generated in the hospital belongs to this category. The safe disposal of this waste is the responsibility of the local authority.

Biomedical Waste

- Deep burial
- Autoclave and microwave treatment
- Shredding
- Secured landfill
- Incineration

SAFETY MEASURES

All the generators of biomedical waste should adopt universal precautions and appropriate safety measures while doing therapeutic and diagnostic activities and also while handling the biomedical waste.

Universal Precautions

- Assume that all patients/specimens are potentially infective for human immunodeficiency virus (HIV)/hepatitis B virus (HBV) and other blood-borne pathogens.

- All blood specimens and body fluids stored should be placed in a leak-proof impervious bag for transportation to the laboratory.
- Use gloves while handling blood, body fluid specimens and other objects disposed to them. If there is likelihood of spattering, use face masks, gloves and goggles.
- Wear laboratory coat or gown while working in the laboratory. Wrap-around gowns should be preferred. These should not be taken outside the laboratory.
- Never try to pipette by mouth. Mechanical pipetting devices should be used.
- Decontaminate the laboratory work surfaces with an appropriate disinfectant after spillage of blood or other body fluids and when the procedures are completed.
- Limit use of needles and syringes to situations for which there are no alternatives.
- All the potentially contaminated material of the laboratory should be decontaminated before disposal or reprocessing.
- Always wash hands after completing work and remove all protective clothing before leaving the laboratory.

Personal Protection

- Immunization against hepatitis B and tetanus shall be given to all hospital staff.
- All the generators of Biomedical waste should adopt universal precautions and appropriate safety measures while doing therapeutic and diagnostic activities and also while handling the biomedical waste.
- All the sanitation workers engaged in the handling and transporting should be made aware of the risks involved in handling the biomedical waste.
- Any worker reporting with an accident/injury due to handling of biomedical waste should be given prompt first aid. Necessary investigations and follow up action as per requirement may be carried out.

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Assess Yourself

LONG ANSWER QUESTION

1. What do you mean by biomedical waste and how do you segregate it?

MULTIPLE CHOICE QUESTIONS

1. Discarded medicine comes in which category of biomedical waste:
 - a. Category 1
 - b. Category 3
 - c. Category 5
 - d. Category 7
2. Human anatomical waste should be treated by:
 - a. Incineration
 - b. Autoclaving
 - c. Microwaving
 - d. Shredding
3. Yellow plastic bag contains wastes of all the categories below except:
 - a. Category 6
 - b. Category 7
 - c. Category 1
 - d. Category 3
4. Red plastic bags should be treated by the following except:
 - a. Incineration
 - b. Autoclaving
 - c. Chemical treatment
 - d. Microwaving
5. Which immunization is necessary for hospital staff?
 - a. DPT
 - b. Hepatitis C
 - c. Hepatitis A
 - d. Hepatitis B

ANSWERS TO MCQS

1. c 2. a 3. b 4. a 5. d

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