ZO (N) 120& ZO (N)-120 L

B. Sc. 1st Semester

Microbiology, Immunology & Biotechnology

DEPARTMENT OF ZOOLOGY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

ZO (N) 120 & ZO (N) 120 L

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Course 4: Practical Zoology
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BLOCK I: MICROBIOLOGY

UNIT 1DIVERSITY OF MICROBES

CONTENT

1.1 OBJECTIVES

- To understand basic study area of Microbiology.
- To study different kinds of microbes like Viruses, Archaea, Bacteria etc.,

1.2 INTRODUCTION

What is Microbiology?

Microbiology is the special branch of biology that deals with the study of microscopic organisms or microorganisms. Microorganisms comprise a large and diverse group of very small organisms that exist as single cells or cell clusters and normally observable only through a microscope. Both prokaryotic and eukaryotic types of microorganisms are found on this earth. In addition to this, non-cellular living structures called viruses are also included in this category.

Individual cell of multicellular eukaryotic creatures is different from microbial cells in the sense that they are unable to live alone in nature and can exist only as constituent part of the body of the larger organism. In contrast to higher organisms, microorganisms are generally able to exhibit essential life features like growth, energy generation and reproduction independently of other cells, either of the same kind or of a different kind.

Distribution of microorganisms in nature

Microorganisms are prevalent nearly everywhere in nature under diverse geographical conditions ranging from the bottom of ocean to the peaks of icy mountains. They can be carried by air currents from the earth's surface to the upper atmosphere. The microbes are found abundantly in places where they find food, moisture and suitable temperature and pH for growth and multiplication.

Impact of microorganisms

Understanding the life processes of microorganism is of huge importance for us. The initial attempts in this direction were limited to the study of disease related pathogenic microorganisms. It was only since the last century, the immense benefits of these tiny creatures in many industrial sectors were realized by mankind. Some microorganisms are

well known to produce antimicrobial compounds in the form of wonder drug called 'antibiotics'. They have also been a source of various other important industrial products like solvents, enzymes, vitamins, growth factors, flavoring products, therapeutic agents etc. Many important steps of our agriculture system depend on microbial activities. Nitrogen fixation is one of the important activities that is carried out by bacteria by forming nodules in association with leguminous plants. Various other plant growth promoting activities of microorganisms like phosphate solubilization, production of siderophores and phytohormones etc. are also well documented and utilized for increased agricultural yield. Microorganisms have an important role in food industry also where they are utilized for manufacturing of products like cheese, yogurt and buttermilk. In addition to this, various alcoholic beverages are also produced by employing them. Microorganisms are also important in the process of waste disposal and energy production. Various types of sewage and waste disposal system have been designed where microorganism are the principal components. In many cases the waste material can also be transformed into some useful products by microorganism.

1.3 BASIC INTRODUCTION TO MICROBIOLOGY

1.3.1. KINDS OF MICROBES

Although microscopic in nature, microorganisms are highly diverse and heterogenic group of organisms. They may belong to various categories depending on their overall morphology, mode of reproduction, nutrition and various other characteristics. Broadly, they belong to different groups like viruses, archaea, Protozoa, fungi, algae and viruses which may further be sub classified into various subgroups.

1.3.2 VIRUSES

Viruses are non-cellular infectious entities having either DNA or RNA as their genome. They cannot grow in artificial media as they lack metabolic machinery of their own for growth and reproduction. They require living host such as plants, animals or other microorganisms to grow and are thus regarded as obligate endoparasites. In 1852, a Russian botanist D.I. Ivanovsky demonstrated that the extract from tobacco plants suffering from mosaic disease retained its infectious nature even when passed through a filter to prevent the passage of

bacteria. Beijerink, in 1898, coined the name ‘virus’ to describe the infectious nature of filtered plant fluids.

Viruses come in an amazing variety of shapes and sizes. They are very small and are measured in nanometers, which is one-billionth of a meter. Viruses can range in the size between 20 to 750nm, which is 45,000 times smaller than the width of a human hair. The majority of viruses cannot be seen with a light microscope because the resolution of a light microscope is limited to about 200nm, so a scanning electron microscope is required to view most viruses.

Characteristics of viruses

- Viruses are the smallest living organisms.
- Unlike other organisms they do not have a cellular structure.
- They can only reproduce by invading living cells by utilizing the metabolic machinery of the host for their own growth.
- Structurally, they just consist of a small piece of nucleic acid, either DNA or RNA, surrounded by a protein or lipoprotein coat.
- They form the boundary between living and non-living objects
- Viruses can have a broad or narrow range of hosts

Virus multiplication

Viruses generally employ a common strategy to replicate. For this they come in contact with their host cell surface and inject their genetic material inside. The genetic material of viruses contains genes for viral coat proteins as well as those for initiating and regulating the viral DNA replication, transcription and translation. Once inside the host cell, the viral genes are expressed and genetic material is replicated many times with the help of the enzymes and cellular machinery of the host cell. Finally many copies of the viral genetic material and coat proteins are manufactured inside the host cells which are further assembled to form numerous new virus particles. These virus particles finally release from the host cell in most of the cases by rupturing and killing it.

Viruses as agents of diseases:

Viruses infect both eukaryotic and prokaryotic cells. Viruses cause a wide range of diseases among plants, animals and fungi. Diseases of humans caused by viruses include measles, chickenpox, influenza, herpes, AIDS, hepatitis, dengue etc. There are evidences that viruses may be the causative agent of some kind of cancers also.

1.3.3- ARCHAEA

Archaea are a domain of single cell prokaryotic organisms that normally inhabit the extreme environment on the earth. Like bacteria they do not have cell nucleus or any other membranous organelles inside their cells. However, they have distinct molecular characteristics separating them from bacteria e.g. archaea cell walls do not contain peptidoglycan like bacteria. They have different membrane lipid composition than bacteria.

Distribution

Archaeans include inhabitants of most extreme environments on the planet. Some live in deep sea while others live in hot spring or in extremely acidic or alkaline environment. They have been found thriving inside the digestive tracts of cows, termites and marine life where they produce methane. They live in the anoxic muds of marshes and at the bottom of the ocean, and even thrive in petroleum deposits deep underground.

Types of Archaea

There are three main types of Archaea:

1) Crenarchaeota- these are characterized by their ability to tolerate extreme in temperature and acidity.
2) Euryarchaeota- these include methane producers and salt lovers.
3) Korarchaeota - a catch-all group for archaeans about which very little is known.

1.3.4- BACTERIA

Bacteria are small, unicellular microscopic organisms approximately 0.5 to1.0 µm in diameter with variable shapes. Bacteria can survive in various extreme conditions because of their ability to reproduce faster and transform into metabolically inert yet live forms called spores. Bacteria are important part of our ecosystem as they carry out various chemical transformations which are essential for sustaining life on the earth. They are the natural

scavengers on the earth which cause decaying of dead and waste matter and hence protect the environment by recycling of elements. Bacteria are important for the survival of both animal and plants. Each animal has a normal bacterial flora in its body particularly in the guts for carrying out processes for complete digestion and degradation of unused food. Plants also depend on the bacterial activity for enhancement of soil fertility. Since last century bacteria are being used industrially for the production of a number of food items, nutrient supplements, medicines, flavouring agents, vaccines, solvents, enzymes, antibiotics etc. Unfortunately, many of them are the causative agents of various animal and plant diseases also.

1.3.5-EUKARYOTIC MICROORGANISMS

The eukaryotic microorganism includes Protozoa (unicellular), fungi and algae (unicellular or multicellular).

PROTOZOA

The protozoa are heterotrophic, single celled, eukaryotic microorganisms with diameter between 5 and 250 micrometers. They are found in almost all moist habitats and commonly present in the sea, soil and freshwater. Members of these groups are free-living as well as parasitic in nature. The locomotors of protozoa include flagella, cilia or Pseudopodia. They reproduce asexually by binary fission, multiple fission or budding and sexually by conjugation. Study of protozoa is important as many of them infect humans and cause various diseases e.g. plasmodium causes malaria in humans.

FUNGI

The fungi (sing. fungus) are a diverse group of eukaryotic heterotrophic microorganisms largely feeding on dead or decaying organic matter. They can also exist as pathogen to plant and animal cells. Fungi are spore-bearing organisms that lack chlorophyll and capable to reproduce by both sexual and asexual modes. Asexual reproduction involves processes like budding, fragmentation, sporulation etc. while sexual reproduction in fungi takes place by means of fusion of compatible nuclei of two parent cells. Several thousand species of fungi have been characterized so far and the number is expected to rise to many millions in future. The habitats of fungi are quite diverse. Some are aquatic, living primarily in fresh water and few in marine habitat. Most are terrestrial and often play crucial roles in mineralization of

organic carbon in nature. Fungi can be broadly classified into two groups i.e. **yeasts** and **molds**. Yeasts are unicellular creatures which resemble bacterial colonies when grown in culture medium. On the other hand, molds are long tangled filaments of cells. These filaments called hyphae (sing., hypha) are intertwined together to form the cotton like structure called mycelium (pl., mycelia). Some fungi are dimorphic that is they exist in two forms.

Fungi are important to us as they break down complex organic compounds, mainly the remains of animal or plant, into simpler compounds that can improve soil fertility. There is a big industrial use of yeasts for making various alcoholic beverages and bakery products. Various filamentous fungi such as Penicillium sp. are being used for the production of antibiotics for treatment of infectious diseases in humans and animals. However, fungi are undesirable at many places as they can decompose timber, textiles, food and other materials. They can also cause various diseases in humans, animals and plants.

Algae (sing., alga) are unicellular or multicellular organisms that contain chlorophyll. They form a heterogeneous group in terms of size, habitat and reproductive processes of the members. The microscopic algae are unicellular and comparable in size to bacteria. Their shape of unicellular algae may be spherical, rod like, club-like or spindle like.

Compared to other green plants algae have simple reproductive structures for sexual reproduction in which a unicellular alga itself may function as a gamete. They can also reproduce asexually by producing flagellated spores and/or non motile spores in sporangia. Algae are important for sustaining life on earth as primary producers of organic matters. They are also source of important products of commercial value such as agar, alginic acid and carrageen a. Many algal species are also being used as food or food supplements in different part of the globe.

1.3.6 TYPICAL STRUCTURE OF BACTERIA

Typical bacterial cell shapes include spherical (coccus, pl.,cocci); straight rods (bacillus, pl.,bacilli) or rods that are helically curved (spiral). Bacterial cell may also exhibit several other types of non-common shapes like, pear shaped, lobes spheres, rods with squared ends etc. Depending on the species, bacterial cells may prefer to stay together in a characteristic pattern or arrangements. For example group of two spherical bacterial cells known as ‘diplococci’, a chain of three or more spherical bacteria as ‘streptococci’ and a group of four

spherical cells as 'tetrad'. Similarly, rod shaped bacterial arrangements are called as 'diplobacilli' and 'streptobacilli' which include two cells or a chain of three or more cells, respectively. Spiral bacteria include vibrios which are curved rodes, spirilla which are helical and rigid and spirochetes which are helical and flexible (Fig.1) Based on the structure of cell wall, bacteria can be broadly classified into two groups i.e. Gram positive and Gram negative. Compared to Gram negative bacteria, Gram positive bacteria have a thick layer of peptidoglycan in their cell wall. On the other hand, Gram negative bacteria have an additional plasma membrane as part of their cell wall which Gram positive bacteria lack. Fig. 2 depicts a typical bacterial cell structure which contains the following components.

1. **Flagellum**- Bacteria possess hair-like, helical appendages called flagella (singular Flagellum) that protrude through the cell wall and confer swimming motility to it. There are three structural parts of a flagellum i.e. basal body, hook and filament. The protein of the filament is known as flagellin. Depending on the bacterial species, a cell may have flagella at one end, at both ends or throughout the cell surface.
2. **Pili** – Pili (singular pilus) are another filamentous appendages which are hollow, non helical, thinner, shorter, and more numerous than flagella. They play no role in motility instead they are primarily required for a process of genetic recombination in bacteria known as conjugation. They are also believed to be involved in attachment of bacteria to its host cell surface.
3. **Capsule**- Some bacterial cells may be surrounded with a viscous substance made of polysaccharide of polypeptide known as 'Capsule'. Capsules serve a number of functions, which include protection against temporary drying by binding water molecules, blocking attachment of bacteriophage, making resistant against phagocyte cells in host body etc.
4. **Cell wall**- Beneath external structures as capsules, sheaths and flagella is the cell wall, a rigid structure that gives shape to the cell. All bacterial cells except Mycoplasma, contain a cell wall covering the cytoplasmic membrane that is made of Peptidoglycan, a polymer, formed by peptidyl cross linkage of linear chains of the alternating units of N-acetyl glucosamine and N- acetylmuramic linked by beta 1, 4 glycosidic bond. This is an insoluble, porous, cross-linked polymer of enormous strength and rigidity. Its main function is to prevent the cell from expanding and bursting because of uptake of water, since most bacteria live in hypotonic environments.
5. **Cytoplasmic membrane** – Beneath the cell wall is the Cytoplasmic membrane, which is approximately 7.5nm thick and is primarily composed of phospholipids and membrane

proteins. The Cytoplasmic membrane serves as a hydrophobic barrier to penetration by most water soluble molecules. However, there are specific proteins embedded in the cytoplasmic membrane which facilitate the passage of small molecules of nutrients and waste products across the cytoplasmic membrane. Various biochemical reactions of respiration and photosynthesis in photosynthetic bacteria take place across the cytoplasmic membrane.

6. **Cytoplasm** –The entire gelly like viscous material covered by the cytoplasmic membrane of a bacterial cell is known as cytoplasm. It contains bacterial chromosome which is a large single piece of supercoiled DNA confined in a region known as nuceloid. Various other macromolecules like ribosomes, m RNA, tRNA etc. are also present in the cytoplasm. Some bacteria also contain plasmid in the cytoplasm.
7. **Plasmid**- Plasmids are extra-chromosomal small, circular, double stranded DNA molecules that can replicate independently. Plasmids often carry genes that may benefit the survival of the organism for example antibiotic resistance.

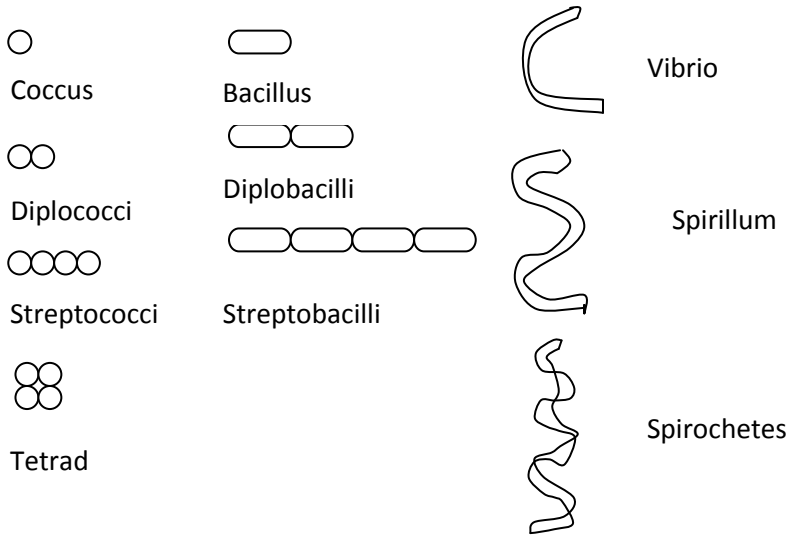


Fig.1.1 Different types of shapes and arrangements of bacterial cells.

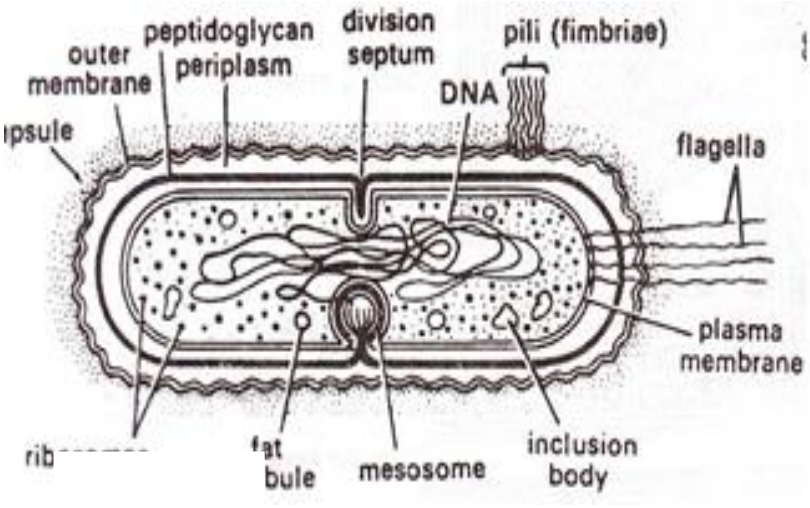


Fig. 1.2 Typical structure of a bacterial cell

1.3.7-TYPICAL STRUCTURE OF VIRUS

A complete virus particle consists of the following parts:

- **Genetic material** – All viruses have a genetic material in the form of either DNA or RNA but never both together. A virus particle may contain single or double stranded form of these nucleic acids.
- **Capsid –** This isprotein coat that surrounds the genetic material and protect it. Capsid is made up of subunits known as capsomeres. A capsid is formed by the repeated joining of various capsomer molecules in a particular pattern. Capsid structure decides the shape or structure of a virus.
- **Envelope-** Some viruses such as HIV and influenza viruses, have an additional lipoprotein layer around the capsid known as envelope. The envelope is made up of a lipid bilayer derived from the cell surface membrane of the host cell. However, it also contains virally encoded proteins which may serve functions like binding to receptors on the host cell or play a role in membrane fusion and cell entry.

A virus structure can be one of the following: helical, icosahedral or complex.

a) Helical

Helical viruses have capsid with a central cavity or hollow tube with nucleic acid in the middle (Fig.3). The capsid is formed by proteins arranged in a circular fashion to create a disc like shapes which are helically attached. They are usually 15-19nm wide and range in length from 300 to 500nm depending on the genome size. Helical viruses may contain an envelope

or not. Examples of enveloped and non enveloped (naked) helical viruses are influenza and tobacco mosaic viruses, respectively.

b) Icosahedral

These viruses are more-or-less spherical in shape involving icosahedral symmetry for bonding and packaging of capsid subunits (Fig.4). The icosahedron is a regular polyhedron with 20 trianglular facets and 12 corners. The genetic material is fully enclosed inside the icosahedrally shaped capsid. Example of enveloped icosahedral viruses is herpes virus whereas poliovirus is a naked virus of this type.

c) Complex

These virus structures have a combination of icosahedral and helical shape and may have a complex outer wall or head-tail morphology. The examples are bacteriophages i.e.viruses that infects bacteria. The head of many bacteriophages has an icosahedral shape with a helical shaped tail (Fig.5). In some bacteriophages whiskers and collars are present at the top of the tail which is required for efficient tail fiber attachment during phage assembly. At the end of the tails there may occur long tail fibers which help bacteriophage attach to the host cell surface.

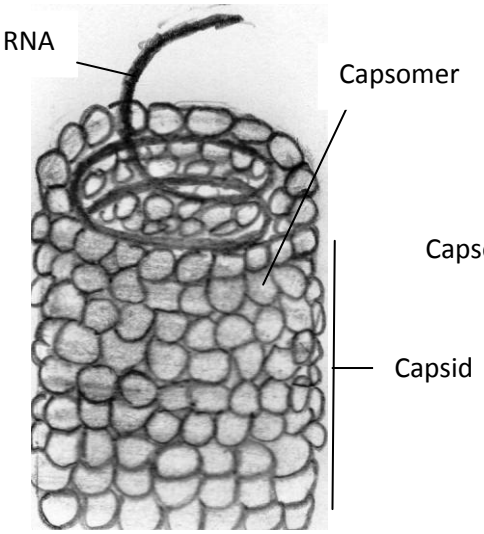


Fig.1.3 Helical virus (TMV)

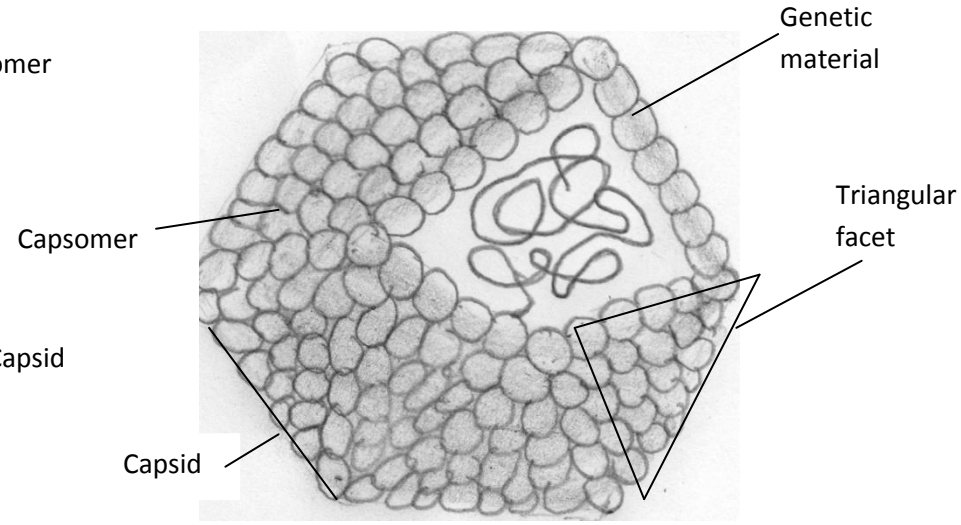


Fig.1.4 Icosahedral virus

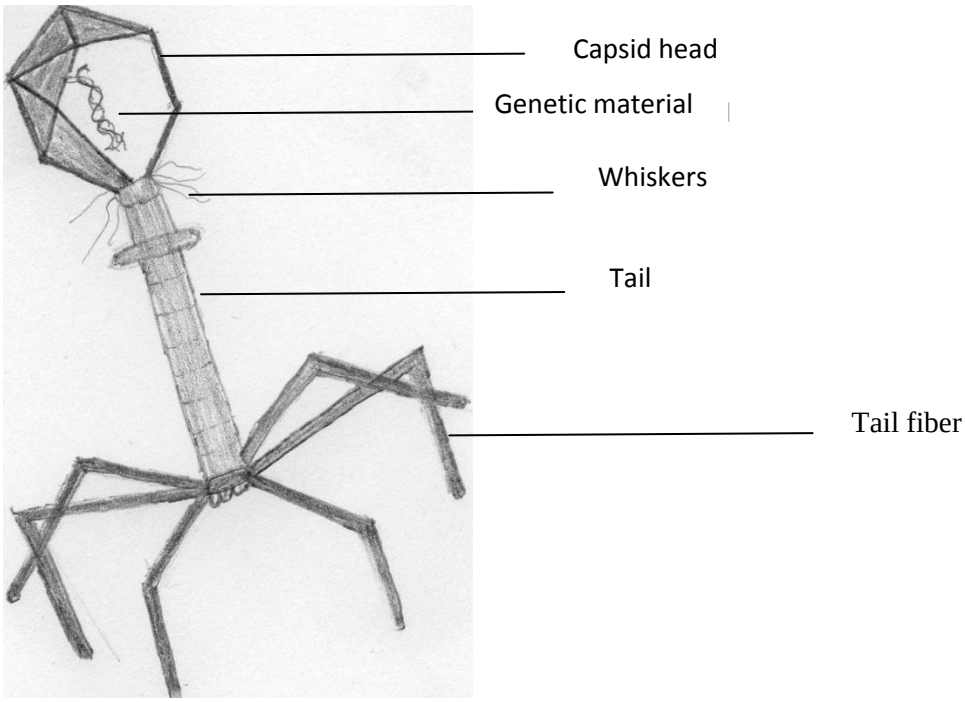


Fig.1.5 Complex virus (Bacteriophage)

1.4 SUMMARY

Microbiology is the study of living organisms which can't be seen in individual form by the naked eyes. These organisms include bacteria, fungi, unicellular algae, protozoa and viruses. Except viruses, all microorganisms have a cellular structure. Depending on their type they may undergo sexual or asexual reproduction or both. Fungi, algae and protozoa are the eukaryotic microorganisms whereas bacteria are prokaryotic. Archae are another class of microorganisms which largely resemble bacteria except few differences. May of them can cause various diseases in humans, animals and plant. However, many of them are very important for their ecological role as well as for medical and industrial applications.

1.5GLOSSARY

1. **Algae:** chlorophyll containing organisms with simple morphological features that range from microscopic and unicellular to very large and multicellular
2. **Archaea**: Bacteria like prokaryotic organisms inhabiting extreme environment
3. **Bacteria:** a group of microscopic organisms that are prokaryotic in nature
4. **Capsid**: Protein cover that surrounds genetic material in a virus
5. **Capsomer**: Protein subunits of a capsid
6. **Flagella**: Locomotory structures of bacteria
7. **Fungi:** a type of eukaryotic microscopic organism that lacks chlorophyll
8. **Pili**: Hairlike structures on the surface of bacteria used for genetic material transfer and host binding
9. **Plasmid**: Extra chromosomal genetic elements found in some bacteria and yeasts
10. **Prokaryotes:** microscopic organisms like bacteria that lacks membrane covered genetic material
11. **Protozoa:** A type of unicellular eukaryotic microscopic organisms
12. **Virus**: Infectious particle made up of nucleic acid, proteins and sometime a proteo-lipidic envelope

1.6SELF ASSESSMENT QUESTIONS

i) Write short notes on the following

a) Bacterial capsule

b) Virus multiplication

c) Plasmid

d) Protozoa

e) Microscopic algae

ii) Describe structure of a typical bacterial cell

iii) Explain different structure of viruses with the help of labeled diagrams

1.7 REFERENCES

1. Madigan MT, Martinko JM and Parker J. Brock Biology of Microorganism 10th edition Pearson Education LTD, London, 2003.

2. Pelczar MJ, Chan ECS and Krieg NR. Microbiology 5th edition Tata McGraw- Hill Publishing Company Limited New Delhi, 1998.

1.7 SUGGESTED READINGS

1. Madigan MT, Martinko JM and Parker J. Brock Biology of Microorganism 10th edition Pearson Education LTD, London, 2003.

2. Pelczar MJ, Chan ECS and Krieg NR. Microbiology 5th edition Tata McGraw- Hill Publishing Company Limited New Delhi, 1998.

1.8 TERMINAL QUESTIONS/ ANSWER

Fill in the blanks

a) Bacteria like organisms that can survive at extreme conditions are known as____

b) Helical appendages on bacterial surface for locomotion are known as _____

c) Unicellular fungi are also called_____

d) The protein subunit of capsid is called_____

e) The source of agar is _____

Answer: a) Archaea b) Flagella or flagellum c) Yeast d) Capsomer e) Algae

State True (T) or False (F)

a) Bacteria are eukaryotic organisms

b) Viruses can infect bacteria also

c) Microscopic algae do not contain chlorophyll

d) Some fungi are the source of antibiotics

e) Plasmodium is a type of protozoa

Answer: a) F b) T c) F d) T e) T

UNIT 2: CULTURE OF MICROBES

CONTENT

2.1- OBJECTIVES

- To learn techniques of microbial sterilization and disinfection
- To learn the techniques involved in laboratory cultivation of microorganisms
- To understand microbial growth
- To learn about the techniques for identification of microorganisms.

2.2- INTRODUCTION

Study of microorganisms is important for us because of their role in human and animal health as well as applications in industries, environment and agriculture. To make study on microorganisms we require them in large number in pure form and this could be possible due to the development of a number of techniques for microbial culture. We are familiar with the cultivation of plants in closed chambers and in a similar way microorganisms can also be grown in the laboratory within various types of culture vessels. As plants need soil full of nutrients for their growth, microorganisms also require nutrients which are provided to them in the form of a culture medium? The unwanted and undesirable kind of microorganisms are destroyed or prevented to grow by various techniques of sterilization and disinfection. Microorganisms can be identified based on their morphological, biochemical and molecular properties.

2.3- BASIC INTRODUCTION: CULTURE OF MICROBES

2.3.1- STERILIZATION

Sterilization means killing, elimination or deactivation of all forms of life including microorganisms from an object. The object can be a surface, avessel, chemical reagents or culture media, a closed chamber etc.The sterilization process is essential in microbiological lab practices to get rid of the unwanted kind of organisms which, if allowed to grow, can result into several undesirable conditions. There are various ways in which sterilization can

be carried out and this mainly includes treatment of **heat, chemicals, irradiation and filtration**.

Heat sterilization can be achieved by applying wet heat, dry heat or by direct exposure to a flame. Under wet heat sterilization, heat is generated by boiling of water under high pressure. In microbiological laboratories this is normally carried out by using a machine called autoclave which is a double-walled cylindrical apparatus made up of thick stainless steel or copper. There is a provision of a lid at one end to open the autoclave and keep the materials to be sterilized within it. A certain amount of water is filled in the outer cylinder and objects to be sterilized are kept in the inner cylinder having holes all around for the free circulation of the steam (Figure 1). There is a heating element at the bottom inner side of the apparatus which can be connected through a cord to the electrical power connection. While operating the machine the lid is closed and the power is switched on that result in the heating of water filled inside. Since the apparatus is tightly closed a pressure also builds up just like within a pressure cooker. A pressure gauge generally fitted at the lid, measures the pressure and an exhaust valve or whistle releases the extra pressure. A safety valve is also placed in the lid to avoid explosion in case the machine is not operating normally and pressure keeps increasing inside. Generally, the laboratory autoclaves are designed to operate at a pressure and temperature of 15 psi and 121^0C, respectively. Under these conditions the autoclave is run for 15 minutes to bring about sterilization.

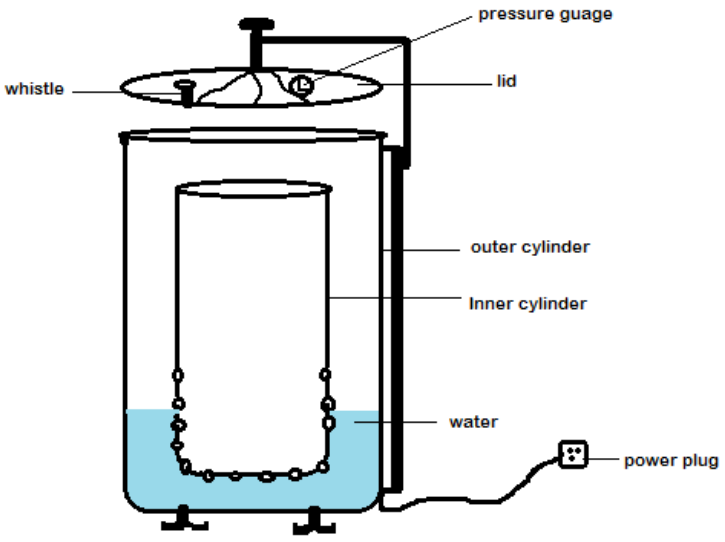


Figure 2.1: An autoclave and its major components

Sterilization using **dry heat/ hot air**takes a longer time compared to wet heat methods. This is suitable for killing a large number of microorganisms and primarily used for sterilization of glasswares such as test tubes, petri plates, beakers, flasks, reagent bottles, glass pipettes, etc, that can tolerate the prolonged exposure to dry heat. Some other heat stable materials including oils, powders, and waxes can also be sterilized using dry heat as they are adversely spoiled by the moist heat of the autoclave. Ovensor hot air ovens as shown in Figure 2 are used for dry heat sterilization. The materials to be sterilized are kept inside the oven where they areexposedto a temperature rangingbetween 150-200 ºCfor 2-4 hours depending upon the type of material to be sterilized. Generally, an oven is operated at 160 ºC for normal sterilization work and glassware gets fully sterilized within 2 hours.

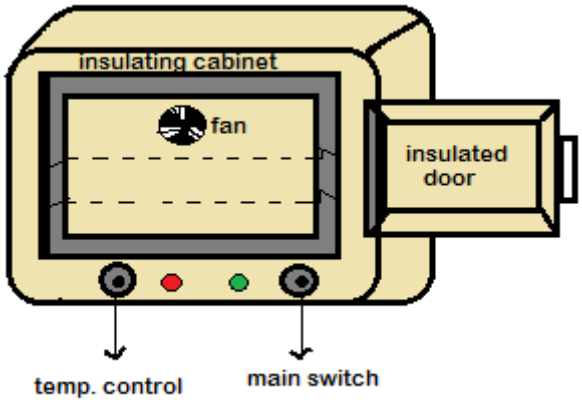


Figure2. 2: Image showing parts of hot air oven

Direct heating on flame (flaming**)**is a method of choice to sterilize inoculating loops, needles and glass rod spreaders. The loop or needle is kept at the flame of Bunsen burner or sprit lamp unless it turns red (Figure 3). This redness of loop ensures the killing ofall microorganisms present on it.

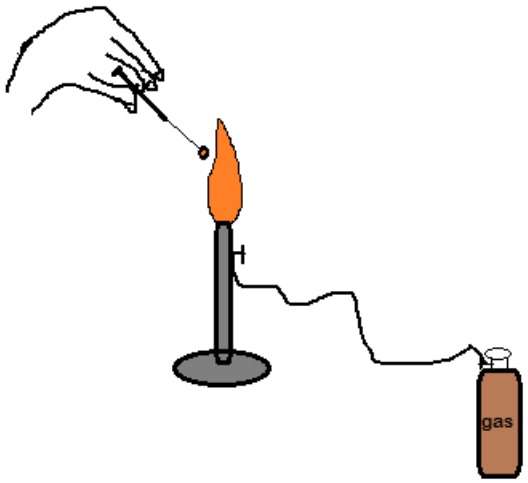


Figure 2.3: Sterilization of inoculating loop on a flame

Tyndallization involves periodic heating upto or slightly below the boiling point of water for sterilization of organic material for prolonged period. The technique is however, occasionally used now days for sterilization of materials which cannot withstand high pressure. On the other hand, Incineration is a method in which organic substances are combusted into ashes. This method is used to sterilize various types of bio-hazardous waste material before discarding.

Although heating provides a reliable way of sterilization but it is not suitable for heat-sensitive materials such as biological compounds, fiber optics, electronics, and many type of plastics. Various chemicals in gas or liquid form can be used to sterilize such heat-sensitive objects. Ethylene Oxide is a gas which is very effective to sterilize a variety of materials and kills almost all known bacteria, yeasts, molds, viruses, spores of bacteria and fungi. But it is inflammable and harmful to human health which restricts its excess use. Nitrogen Dioxide can kill a wide range of microbes including spores viruses and common bacteria and therefore, efficiently used for sterilization. Among liquid chemicals, ethyl alcohol is commonly used for surface sterilization of a variety of objects. Liquid and vaporized hydrogen peroxide is another sterilizing agent that has a strong oxidant activity to destroy a wide range of pathogens. The main advantage of H_2O_2 as sterilants is the short time about 28 minutes. In addition to these, Glutaraldehyde and formaldehyde, although commonly used as fixatives, are also accepted as liquid sterilizing agents with longer immersion time.

Radiation is also used for sterilization purposes in microbiology. Gamma rays or ultraviolet rays (UV) are commonly used for this purpose. Gamma rays have very short wave length and high energy and due to that they have high penetrating power and are very lethal to living organisms. This is used for sterilization of materials of considerable thickness and volumes, e.g. plastic wares, packaged foods and medical devices. On the other hand, UV light has a very little penetrating power and therefore can be used for killing of microorganisms on the surface of an object by way of direct exposure to it.

Bacteriological filters are also used for removal of bacterial cells from a liquid material. Although it is not a complete sterilization process but for practical purposes it is very useful and sufficient for preventing the bacterial contamination. Filters for this purpose, called membrane filters, are commercially available and the solution just needs to be filtered under aseptic conditions. The solutions of heat labile compounds such as antibiotics, vitamins etc. are filter sterilized instead of autoclaving.

2.3.2-DISINFECTION

Disinfection is a process in which harmful microorganism are removed from non-living objects or surfaces. Like sterilization, disinfection also renders an environment contamination free. However, unlike sterilization, in disinfection complete removal of all life forms does not occur as endospores are left intact. Disinfection is frequently required in hospitals, surgical theatres, specialized laboratories, bathrooms, kitchens etc. Disinfection is achieved by the use of certain antimicrobial agents known as disinfectants. Disinfectants are of two types i.e. oxidizing and non-oxidizing. Among oxidizing disinfectants sodium hypochlorite, iodine, hydrogen peroxide are commonly used. Oxidizing agents react with a variety of essential biomolecules within the microbial cell and renders them inactive as a result the organism dies. The oxidizing agents can break the covalent bonds in DNA, RNA and proteins as well as break down lipids into smaller fatty acids. The non-oxidizing disinfectants are also known as coagulating agents that include alcohol, quaternary ammonium compounds, phenol, and glutaraldehyde and ethylene oxide. Action of these non-oxidizing agents on microbial cells results in the cross-linking and coagulation of nucleic acids, proteins and amino acids. The microbial cell can no longer survive due to the inactivation of these key biomolecules.

2.3.3- CULTURING

In microbiology, culturing refers to the cultivation of microorganisms by providing suitable growth conditions in the laboratory. Like other organisms, microbes also require nutrients for their growth. The nutrient molecules are metabolized by microbial cell to carry out various cellular processes to survive and grow. For microbial culturing such nutrients are provided to microorganisms in the form of a medium (plural; media). There can be various different type of media which support the growth of different or common group of microorganisms used for general or specialized purpose. A medium is essentially a mixture of nutrients provided to microorganisms in solid, liquid or semi solid form to allow them to grow or increase their number. After growth, numerous microbial cells are produced on the medium and this is collectively known as a microbial culture. In mixed culture there can be many different types of microorganisms having variation at species, genera or other taxonomic level. On the other hand, pure culture describes a culture in which all the cells are exactly similar to each other pure culture is established by group of cells which are the descendents of the same parental cell.

As stated above, in a culture microbial population is maintained on a medium. Under routine practices in the laboratory a microbial culture is established in solid or liquid medium. The solid medium is kept within a culture tube normally in a slanted position called a ‘slant’ or within round shaped plates known as petri plates. Microbial culture either as a colony, streak or mat is maintained on the surface of the solid media within the culture vessels.

2.3.4- MEDIA PREPARATION

The food material which is needed by the microorganisms for their growth in the laboratory is regarded as culture medium. The growth of a microbe on the medium itself is called a culture. So the culture medium is a composition with ingredients being various nutrients like inorganic and organic compounds onto which a population of microorganisms (culture) is grown under suitable conditions in the laboratory. It is used to grow, transfer and store microorganisms. Although all microorganisms have almost similar basic molecular requirements but there may prefer various different organic and inorganic compounds as their source. Hence, different types of culture media are used to cultivate different types of

microorganisms. In addition to this, theyare also used for identification, enumeration and maintenance of microbial cultures.

Media may be classified into various types like selective media, differential media, maintenance media, enumeration media, media for microbial characterization etc. Selective media are prepared with nutrients which are favourable for the growth of a particular type of microorganisms. The other types are either not supported or their growth is inhibited. For example a medium in which starch is the only source of carbon will be selective for the growth of microorganism which can produce an enzyme 'amylase' for its digestion. Differential media are prepared to distinguish between different groups of microorganisms based on the difference in their growth pattern which can be visually detected. For example, the medium blood agar can be used for differentiating between haemolytic and non-haemolytic bacteria based on the presence of clear haemolytic zone around the bacterial colony on this medium. Maintenance media are developed for maintaining the culture for a longer time by growth under suboptimum conditions. This can be achieved by omitting the rapidly utilizable compounds such as glucose in the medium. Enumeration media are those used for enumeration of the bacterial population of material like milk, water etc. The biochemical properties of a microorganism can be detected by preparing appropriate media for its characterization and identification.

For preparing a medium, first of all, the ingredients of it are determined and accurately weighted and mixed together in water. The pH is also set as per the requirement. To prepare a solid medium, agar, a complex polysaccharide, is also added to it. The whole mixture is sterilized usually in an autoclave. The heat labile compounds such as vitamins, antibiotics etc. can be separately sterilized generally by filtration and added to it under aseptic conditions. The medium is then poured within a Petri plate or a culture tube where due to the gelling properties of agar the whole mixture solidifies as temperature cools down. In case the medium is required in liquid form, no agar is added and it is preferably kept within conical flasks or culture tubes after sterilization.

2.3.5- ISOLATION

In microbiology, isolation is defined as a method of separating and growing specific microbial types from a mixed population. The sources of this mixed population can be various environments such as soil, water, air, food material, milk products, animal body including humans etc. To isolate a microbial culture from any of these environments, first an

appropriate solid or liquid medium is prepared and sterilized. The environmental sample is then brought in contact with the medium. For this, usually the sample with or without dilution is added to the liquid medium or plated or streaked on the solid medium surface. The microbial growth on the liquid medium turns it turbid whereas on the solid medium the microbial growth can be observed as a colony, mat or in a linear pattern in which the sample was streaked on it. In both cases, the microbial mass can arise due to the growth of a mixed population. Out of it pure cultures can be prepared by repeated streaking on the media plates. An example is illustrated in Figure 4 to show the process of bacterial culture isolation from a sample. Here, a known amount of sample i.e. 1gm or 1 ml is first mixed with 9 ml of sterile distilled water to make a final volume of 10 ml.Serial dilutions are made by transferring 1ml of this to 9 ml of sterile distill water and the process is repeated to get fold dilutions as 10^{-1}, 10^{-2}, 10^{-3} etc. Number of dilutions to be made depends upon the microbial load of the sample used. For example, samples with high microbial load such as animal feces require more dilutions before plating to obtain individual colonies of microbes.

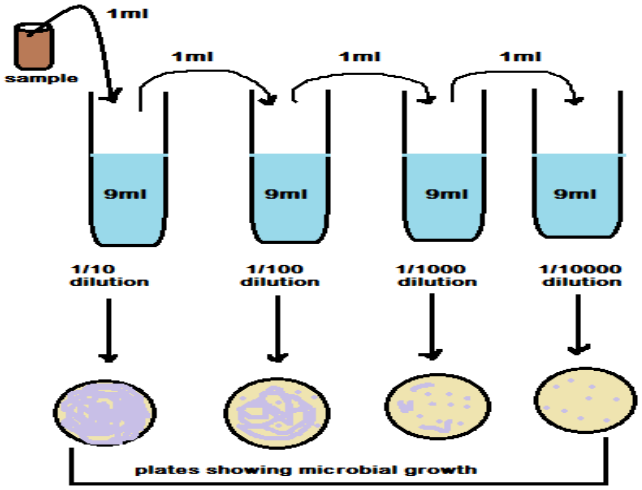
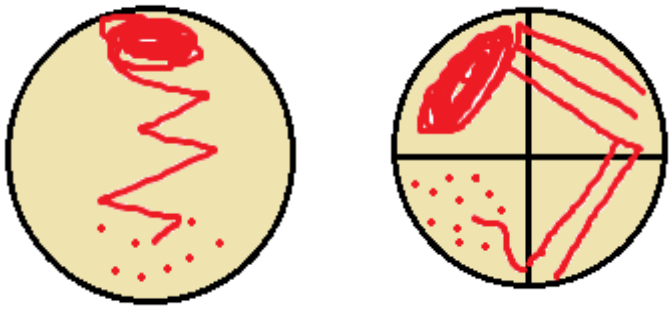


Figure 2.3: Serial dilution and plating of a sample

From each of the serially diluted sample a small amount generally 50-100 µl istransferred (called inoculation) on the surface of solid media within sterile Petri plates. The sample is then uniformly spread on the surface with the help of a sterile spreader (L-shaped glass rod).The plates are then kept inverted for incubation at a particular temperature and time duration depending on the preferred growth conditions of the microorganism used. For most of the bacteria, incubation at 37^0C for 24 -28h is sufficient. The microbial growth on the plate can be observed after the incubation.

For obtaining a pure culture, e.g. bacteria as obtained in the form of a colony in the previous step can be repeatedly streaked on the petri plate containing media in order to get a pure culture of the same (Figure 5a &b)



(a) (b)

Figure 2.4: Bacterial growth on petri plate after streaking

2.3.6- GROWTH AND IDENTIFICATION OF MICROORGANISM

Microbial growth is generally defined as the orderly increase in the population of microbial cells. Certain physical and environmental factors influence the microbial growth, which mainly include temperature, pH, osmotic pressure, nutrient availability, presence / absence of oxygen etc.Bacteria grow and increase their number in an environment by the process of cell division of a parental cell (Figure 6). Transverse binary fission is the most common and most important mode of cell division in bacteria. In binary fission, one microbial cell divides into two physically and genetically similar daughter cells by developing a transverse septum, also known as a cross wall. The other modes of cell division are budding, fragmentation etc. The daughter cells thus produced, divide further in a similar manner and it goes on for many generation to form a large bacterial population.

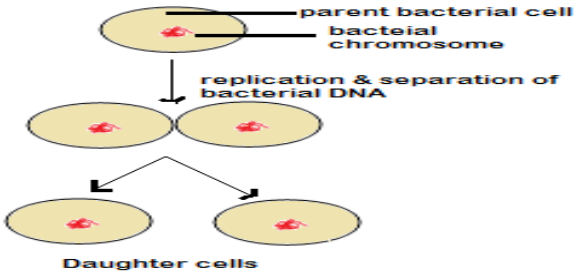


Figure 2.5: Bacterial cell division and growth

In this manner if we consider N_0 number of bacterial cells divide and grow for a time period T in which 'n' number of division occurs to give a population of N numbers of cells, the following expression can be defined.

$N=N_0X\ 2^n$

This can be reorganized as $n= 3.3\ (\log_{10}N-\log_{10}N_0)$

The generation time G i.e the time required for the population to double in number can be calculated by the following formula

$G=T/n=T/3.3\ (\log_{10}N-\log_{10}N_0)$

If we periodically measure bacterial growth by determining the number of cells in a medium and plot against time we shall get a bacterial growth curve that will be very helpful in understanding different stages of growth. A typical bacterial growth with different stages of growth is shown in Figure 7.1

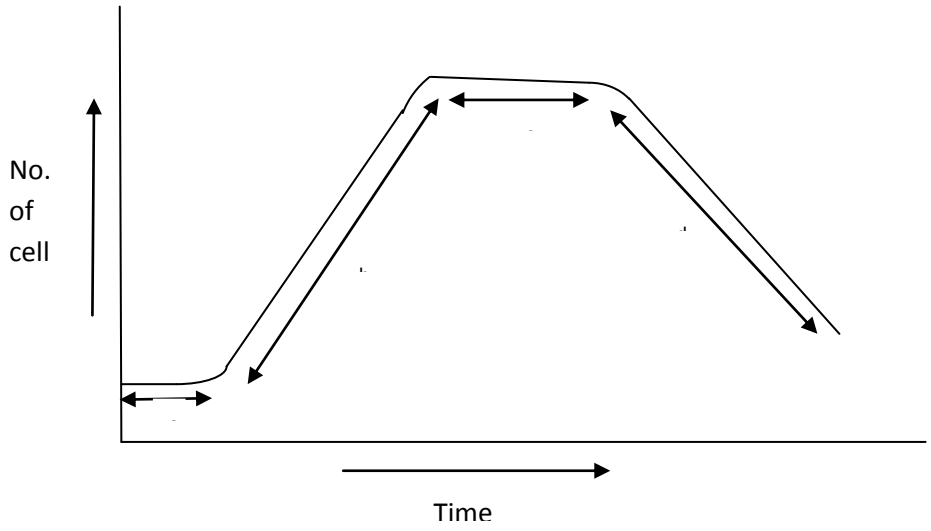


Figure 2.6: Bacterial growth curve

Four distinct bacterial growth stages can be identified in a bacterial growth curve as depicted in Figure 7 as a, b, c &d. The first stage 'a' is Lag phase and it denotes the period of acclimatization. During this period microbes present in the inoculum adjust them to the new environment. Although cells are metabolically very active and grow in size but do not divide at this stage. This is followed by Logarithmic phase or Exponential phase (Stage b). This is the phase of rapid division and growth. During this phase only the mathematical expressions of bacterial growth as mentioned above are effective. The microbial population reaches at its maximum at the end of this phase. Thereafter, further increase in the population does not occur with time and the microorganisms enter the stationary phase (Stage c). This phase

arises due to many reasons the most prominent being the depletion of a key nutrient or accumulation of some toxic compounds. The population remains constant during this stage and after this 'death phase' (Stage d) prevails in which rapid decrease in the number of bacterial cells occurs.

2.3.7- IDENTIFICATION OF MICROORGANISMS

Microorganisms particularly bacteria have been classically identified by examining their morphological and biochemical features and staining characteristics. The immunological reactions of their surface antigens have also been used to classify them. Nowadays with the advent of molecular techniques, DNA sequence based phylogenetic characterization of microorganisms has become an essential tool in microbial taxonomy. For bacteria, the sequence of 16S rRNA gene is accepted worldwide for species characterization of bacteria. This is also being used for characterizing other higher taxonomic levels such as genera, family, order, class and phylum. Before declaring a bacterial new species, techniques to determine 'percentage G+C content', DNA-DNA cross hybridization, FAME (fatty acid methyl ester) analysis are also employed.

2.4- SUMMARY

Due to the immense importance of microorganisms their routine cultivation in the laboratories is very essential these days. We can establish and maintain microbial culture by knowing some basic techniques. The favourable environment for the desired microbes, which we wish to cultivate, can be created by destroying or preventing the growth of undesired types by sterilization and disinfection. For microbial culture in the laboratory microbes are provided with their food requirements in the form of media. Several kinds of media can be prepared depending on the type of microorganisms. The microorganisms from a sample like soil, water, milk etc. can be grown on a medium and pure cultures comprising a single type of microorganism can be isolated. While establishing a culture on a medium, microorganisms increase their number by division of the parental cells and this is called growth. Various stages of bacterial growth in a liquid medium can be represented by a bacterial growth curve. The microbes can be identified by conventional techniques of morphological and biochemical characterization. The advent of molecular techniques has further refined the process of microbial identification.

2.7- REFERENCES

1. "Division of Oral Health - Infection Control Glossary". U.S. Centres for Disease Control and Prevention. Archived from the original on 13 April 2016. Retrieved 19 April 2016.
2. "Hospital Disinfectants for General Disinfection of Environmental Surfaces" (PDF). *New York State Department of Health. Archived from* the original (PDF) *on 24 September 2015*. Retrieved 19 April 2016.
3. "WHO Glossary".
4. Aneja K.R (2003), Experiments in Microbiology, Plant Pathology and Biotechnology, 4th revised edition; New Age International (P) Limited Publishers.
5. CRC Handbook of Chemistry and Physics, 76th Ed. 1995
6. Frerichs, Ralph R. "Definitions". www.ph.ucla.edu.
7. Kumar P (2014), Life Science: Fundamentals and practice, 4th edition; Pathfinder Publication.
8. Pelczar MJ , Chan ECS , Krieg NR 1993. Microbiology Fifth Edition, TataMcGraw Hill, New Delhi
9. Hong Yoo-J. 2018. Review of disinfection and sterilization-back to the basics. Infection and Chemotherapy, 50(2): 101-109

Block II: IMMUNOLOGY

UNIT: 3 OVERVIEW OF IMMUNE SYSTEM

CONTENT

3.1 OBJECTIVES

- To describe the concepts of innate immunity and acquired immunity
- To list the types of innate immunity and acquired immunity
- To explain the mechanism of innate immunity
- To explain the differences between active and passive immunity

3.2 INTRODUCTION

Immunology is "a branch of science that covers the investigation of safe frameworks in all living beings" by **Janeway** (**2001)**. Immunology is the examination of the immune system and is a basic branch of the helpful and natural sciences. The safe framework shields us from infection through various lines of watch. If the resistant framework isn't functioning as it should, it can realize disease, for instance, autoimmunity, excessive touchiness and tumor. It is similarly now winding up clear that insusceptible reactions add to the headway of various fundamental issue not by and large observed as immunologic, including metabolic, cardiovascular, and neurodegenerative conditions, for instance, Alzheimer's.

3.3 IMPORTANCE OF IMMUNOLOGY

From Edward Jenner's leading work in the 18^{th} Century that would finally provoke immunization in its present day shape (an advancement that has likely secure a greater number of lives than some other helpful advance), to the various legitimate breakthroughs forward in the nineteenth and twentieth many years that would incite, notwithstanding different things, safe organ transplantation, the recognizing verification of blood get-togethers, and the now inescapable usage of monoclonal antibodies all through science and social protection, immunology has changed the substance of current arrangement. Immunological research continues extending horizons in our cognizance of how to treat basic restorative issues, with advancing exploration tries in immunotherapy, safe framework diseases, and antibodies for rising pathogens, for instance, Ebola. Pushing our appreciation of key immunology is essential for clinical and business application and has supported the revelation of new diagnostics and solutions to manage a wide group of diseases. Despite the above, joined with impelling development, immunological research has given fundamentally

basic research techniques and mechanical assemblies, for instance, stream cytometry and balancing specialist advancement.

3.4. HISTORY AND ORIGIN

In **1798, Edward Jenner** built up the first run through **vaccination** process, for smallpox issues. His work was great to the point, that several of individuals respected him for his decision that vaccination with **cowpox** (identified with cow like steers) could offer resistance to **smallpox**. Like this, the idea of **inoculation** (in Latin 'vacca' signifies 'bovine') was begun. In **1878, Louis Pasteur** inadvertently used a debilitate chicken cholera culture and watched, that the debilitated shape spared the chickens from the destructive type of ailment. Later on a contender of Pasteur, **Robert Koch** was the first to isolate the Bacillus anthracis living being and, insensible of Pasteur's work, he could show that it caused the medical problem. By then in 1882, Koch could show that the germ hypothesis of sickness associated with human ailments and also animals, when he isolated the microorganism that caused **tuberculosis**. His "Koch's proposes" are up 'til now used to recognize infective living things.

3.5 IMMUNE SYSTEM

The immune organization is a reticule of tissues, organs and cells that work along to protect the body against assaults by “stranger” intruders. These are fundamentally microorganisms(germs),small, disease-causing living beings,for example, viruses, bacteria, fungiand parasites as the human body gives a perfect situation to numerous microorganisms. The insusceptible framework is incredibly intricate. It can perceive and recollect a large number of various foes, and it can create discharges and cells to coordinate with and wipe out every last one of them. The way to its prosperity is a detailed and dynamic correspondence arrange. Millions of cells, sorted out into sets and subsets, accumulate like billows of honey bees swarming around a hive and pass data forward and backward. When resistant cells get the caution, they experience strategic changes and start to create effective chemicals. These substances enable the cells to manage their own particular development and conduct, enroll their colleagues, and direct newcomers to inconvenience spots. The way to a solid safe framework is its capacity to recognize the body's own particular cells—self—and outside cells—non self. The body's invulnerable barriers regularly exist together gently with cells that convey particular "self" marker atoms. In any case, when invulnerable protectors experience cells or living beings conveying markers that say "remote," they rapidly dispatch an assault.

Anything that can trigger this safe reaction is called an antigen. An antigen can be a microorganism, for example, an infection, or even a piece of an organism. Tissues or cells from someone else (with the exception of an indistinguishable twin) additionally convey non self markers and go about as antigens. This clarifies why tissue transplants might be rejected. In unusual circumstances, the safe framework can mix up self for non self and dispatch an assault against the body's own particular cells or tissues. The outcome is called an immune system malady. A few types of joint pain and diabetes are immune system sicknesses. In different cases, the resistant framework reacts to an apparently safe remote substance, for example, ragweed dust. The outcome is hypersensitivity, and this sort of antigen is called an allergen. Practically, a resistant reaction can be separated into two related exercises acknowledgment and reaction. Insusceptible acknowledgment is astounding for its specificity.

3.6 THE STRUCTURE OF THE IMMUNE SYSTEM

Immunity the condition of security from irresistible sickness has both a not so much particular but rather more particular segment. Immune system is a standout amongst the most critical systems of the body that is vital for human existence. It includes tissues and cells that are related with the protection of our body from various pathogens and irresistible operators. The resistance (or ability to battle an irresistible operator without delivering the indications of sickness) is by and large arranged into two unique sorts: to be specific natural invulnerability and versatile insusceptibility.

3.6.1 INNATE IMMUNITY

The innate immune system, otherwise called the non-particular insusceptible framework or in-conceived resistance framework, is a vital subsystem of the general safe framework. Intrinsic invulnerability alludes to nonspecific resistance components that become possibly the most important factor quickly or inside hours of an antigen's appearance in the body. These components incorporate physical obstructions, for example, skin, chemicals in the blood, and safe framework cells that assault remote cells in the body. The intrinsic safe reaction is enacted by compound properties of the antigen The less particular segment, natural insusceptibility, gives the primary line of guard against contamination. Most segments of intrinsic insusceptibility are available

before the beginning of contamination and constitute an arrangement of infection protection systems that are not particular to a specific pathogen but rather that incorporate cell and atomic segments that perceive classes of particles impossible to miss to as often as possible experienced pathogens. Phagocyte cells, for example, macrophages and neutrophils, hindrances, for example, skin, and an assortment of antimicrobial mixes blended by the host all assume imperative parts in intrinsic insusceptibility. Intrinsic invulnerable framework is additionally alluded to as quick reaction resistant framework. This framework actuates inside minutes to hours after an outside operator attacks inside the human body. Natural safe framework is made out of two lines of barriers.

The significant elements of the intrinsic insusceptible framework include:

- Recruiting invulnerable cells to locales of disease, through the generation of substance factors, including specific compound middle people, called cytokines.
- Activation of the supplement course to distinguish microscopic organisms, actuates cells, and advances leeway of neutralizer buildings or dead cells.
- Identification and evacuation of remote substances show in organs, tissues, blood and lymph, by particular white platelets.
- Activation of the versatile insusceptible framework through a procedure known as antigen introduction.
- Acting as a physical and compound hindrance to irresistible specialists.
  Intrinsic invulnerability can be believed to contain these sorts of cautious hindrances: anatomic, physiologic, phagocytic, supplement framework and incendiary.

PHYSICAL AND ANATOMIC BARRIERS:The physiologic and anatomic obstructions that add to natural insusceptibility incorporate temperature, pH, and different dissolvable and cell related atoms. These keep the passage of pathogens in a creature's initially line of safeguard against disease. The skin and the surface of mucous films are incorporated into this class since they are viable boundaries to the passage of generally microorganisms. The skin comprises of two unmistakable layers: a more slender external layer—the epidermis—and a thicker layer—the dermis. The

epidermis contains a few layers of firmly pressed epithelial cells. The external epidermal layer comprises of dead cells and is loaded with a waterproofing protein called keratin. The dermis, which is made out of connective tissue, contains veins, hair follicles, sebaceous organs, and sweat organs. The sebaceous organs are related with the hair follicles and create a slick emission called sebum. Sebum comprises of lactic corrosive and unsaturated fats, which keep up the pH of the skin in the vicinity of 3 and 5; this pH hinders the development of generally microorganisms. The skin may likewise be entered by gnawing creepy crawlies (e.g., mosquitoes, parasites, ticks, insects, and sand flies); if these harbor pathogenic life forms, they can bring the pathogen into the body as they nourish. The conjunctivae and the wholesome, respiratory, and urogenital tracts are lined by mucous films, not by the dry, defensive skin that covers the outside of the body. These films comprise of an external epithelial layer and a fundamental layer of connective tissue. Various nonspecific resistance systems have a tendency to forestall section of pathogens for instance, salivation, tears, and mucous discharges act to wash away potential trespassers and furthermore contain antibacterial or antiviral substances.

INFLAMMATORY BARRIERS: Tissue harm caused by an injury or by an attacking pathogenic microorganism prompts an unpredictable grouping of occasions all things considered known as the provocative reaction. Aggravation is one of the main reactions of the resistant framework to contamination or disturbance. Irritation is animated by substance factors discharged by harmed cells and serves to build up a physical boundary against the spread of disease, and to advance mending of any harmed tissue following the freedom of pathogens. The incendiary reaction is portrayed by the accompanying side effects:

- redness of the skin, because of privately expanded blood dissemination
- heat, either expanded neighborhood temperature, for example, a warm looking about a restricted disease, or a fundamental fever
- swelling of influenced tissues, for example, the upper throat amid the normal icy or joints influenced by rheumatoid joint inflammation
- increased creation of bodily fluid, which can cause side effects like a runny nose or a beneficial hack
- pain, either nearby torment, for example, excruciating joints or a sore throat,

or influencing the entire body, for example, body hurts

- possible brokenness of the organs or tissues included

The inflammation process is started by cells effectively show in all tissues, essentially occupant dendritic cells, macrophages, Kupffer cells, mastocytes and histiocytes. These cells exhibit receptors contained at first glance or inside the cell, named pattern recognition receptors (PRRs), which perceive atoms that are comprehensively shared by pathogens however recognizable from have particles, aggregately alluded to as pathogen-associated molecular patterns (PAMPs). At the beginning of a contamination, consume, or different wounds, these cells experience enactment (one of their PRRs perceives a PAMP) and discharge incendiary middle people in charge of the clinical indications of aggravation. Synthetic elements created amid irritation are histamine, bradykinin, serotonin, leukotrienes, and prostaglandins which sharpen torment receptors, causing nearby vasodilation of the veins, and pull in phagocytes, particularly neutrophils. Neutrophils at that point trigger different parts of the resistant framework by discharging factors that summon extra leukocytes and lymphocytes. Cytokines delivered by macrophages and different cells of the intrinsic invulnerable framework intervene the incendiary reaction. These cytokines incorporate TNF, HMGB1, and IL-1. The "four cardinal indications of irritation" are rubor (redness), tumor (swelling), calor (warmth), and dolor (torment). The cardinal indications of aggravation mirror the three noteworthy occasions of an incendiary reaction.

VASODILATION - an expansion in the measurement of veins of adjacent capillaries exists as the vessels that divert blood from the influenced region choke, bringing about engorgement of the capillary system. The engorged vessels are in charge of tissue redness (erythema) and an expansion in tissue temperature.

An expansion in capillary penetrability encourages a flood of liquid and cells from the engorged vessels into the tissue. The liquid that collects (exudate) has a significantly higher protein content than liquid regularly discharged from the vasculature. Aggregation of exudate contributes totissue swelling (edema).
Influx of phagocytes from the vessels into the tissues is encouraged by the expanded porousness of the vessels. The resettlement of phagocytes is a multistep procedure

that incorporates adherence of the cells to the endothelial mass of the veins (margination), trailed by their displacement between the narrow endothelial cells into the tissue (diapedesis or extravasation), and, at long last, their relocation through the tissue to the site of the intrusion (chemotaxis). As phagocytic cells aggregate at the site and start to phagocytose microorganisms, they discharge lytic proteins, which can harm adjacent solid cells. The aggregation of dead cells, processed material, and liquid structures a substance called discharge. The final product of aggravation might be the marshaling of a particular resistant reaction to the attack or leeway of the trespasser by segments of the natural invulnerable framework. Once the fiery reaction has died down and the vast majority of the garbage has been gathered up by phagocytic cells, tissue repair and recovery of new tissue starts. Vessels develop into the fibrin of blood coagulation. New connective tissue cells, called fibroblasts, supplant the fibrin as the coagulation breaks up, as fibroblasts and vessels amass, scar tissue is framed.

COMPLEMENT SYSTEM: The complement system is a biochemical cascade of the immune system that helps, or "complements", the ability of antibodies to clear pathogens or mark them for destruction by other cells. The cascade is composed of many plasma proteins, synthesized in the liver, primarily by hepatocytes. The complement system facilitates following actions:

- trigger the recruitment of inflammatory cells
- "tag" pathogens for destruction by other cells by opsonizing, or coating, the surface of the pathogen
- form holes in the plasma membrane of the pathogen, resulting in cytolysis of the pathogen cell, causing the death of the pathogen
- rid the body of neutralised antigen-antibody complexes.

PHAGOCYTIC BARRIERS/CELLS OF THE INNATE IMMUNE RESPONSE:Another critical innate protection component is the ingestion of extracellular particulate material by phagocytosis. Phagocytosis is one sort of endocytosis, the general term for the take-up by a cell of material from its condition. In phagocytosis, a phone's plasma layer grows around the particulate material, which may incorporate entire pathogenic microorganisms, to shape vast vesicles called

phagosomes. Most phagocytosis is directed by particular cells, for example, blood monocytes, neutrophils, and tissue macrophages. Most cell sorts are equipped for different types of endocytosis, for example, receptor-intervened endocytosis. The inborn cells incorporate Natural executioner cells, pole cells, eosinophils, basophils; macrophages, neutrophils, and dendritic cells, which work inside the invulnerable framework by distinguishing and wiping out pathogens that may cause contamination.

MAST CELLS: These cells are a sort of natural insusceptible cell that dwells in connective tissue and in the mucous films. They are personally connected with wound mending and barrier against pathogens, but on the other hand are frequently connected with sensitivity and anaphylaxis. When enacted, pole cells quickly discharge trademark granules, rich in histamine and heparin, alongside different hormonal arbiters and chemokines, or chemotactic cytokines into nature. Histamine widens veins, causing the trademark indications of aggravation, and enlisted people neutrophils and macrophages.

MACROPHAGES: Macrophage from the Greek, signifying "substantial eaters," are extensive phagocytic leukocytes, which can move outside of the vascular framework by moving over the dividers of narrow vessels and entering the territories between cells in quest for attacking pathogens. In tissues, organ-particular macrophages are separated from phagocytic cells introduce in the blood called monocytes. Macrophages are the most effective phagocytes and can phagocytose considerable quantities of microscopic organisms or different cells or microbes. The official of bacterial particles to receptors on the surface of a macrophage triggers it to inundate and devastate the microorganisms through the age of a "respiratory burst", causing the arrival of responsive oxygen species. Pathogens likewise fortify the macrophage to deliver chemokines, which summon different cells to the site of disease.

NEUTROPHILS: Neutrophils alongside two other cell sorts (eosinophils and basophils), are known as granulocytes because of the nearness of granules in their cytoplasm, or as polymorphonuclear cells (PMNs) because of their unmistakable lobed cores. Neutrophil granules contain an assortment of dangerous substances that slaughter or restrain development of microscopic organisms and growths. Like

macrophages, neutrophils assault pathogens by actuating a respiratory burst. The fundamental results of the neutrophil respiratory burst are solid oxidizing operators including hydrogen peroxide, free oxygen radicals and hypochlorite. Neutrophils are the most rich sort of phagocyte, regularly speaking to 50-60% of the aggregate coursing leukocytes, and are generally the main cells to land at the site of a contamination. The bone marrow of an ordinary sound grown-up produces more than 100 billion neutrophils for each day, and more than 10 times that numerous every day amid intense aggravation.

DENDRITIC CELLS (DCS): Dendritic cells are phagocytic cells show in tissues that are in contact with the outside condition, for the most part the skin (where they are regularly called Langerhans cells), and the inward mucosal covering of the nose, lungs, stomach, and digestive organs. They are named for their similarity to neuronal dendrites, however dendritic cells are not associated with the sensory system. Dendritic cells are vital during the time spent antigen introduction, and fill in as a connection between the intrinsic and versatile insusceptible frameworks.

BASOPHILS AND EOSINOPHILS: Basophils and eosinophils are cells identified with the neutrophil. At the point when enacted by a pathogen experience, histamine-discharging basophils are vital in the protection against parasites and assume a part in hypersensitive responses, for example, asthma. Upon actuation, eosinophils emit a scope of exceptionally lethal proteins and free radicals that are profoundly compelling in murdering parasites, however may likewise harm tissue amid an unfavorably susceptible response. Enactment and arrival of poisons by eosinophils are, in this manner, firmly directed to keep any improper tissue pulverization.

NATURAL KILLER CELLS (NK CELLS):NK cells are a segment of the inborn resistant framework that does not specifically assault attacking organisms. Or maybe, NK cells devastate traded off host cells, for example, tumor cells or infection contaminated cells, perceiving such cells by a condition known as "missing self." This term depicts cells with anomalous low levels of a phone surface marker called MHC I (real histocompatibility complex) - a circumstance that can emerge in viral diseases of host cells. They were named "normal executioner" due to the underlying thought that they don't require enactment with a specific end goal to murder cells that

are "missing self." For some years, it was vague how NK cell perceive tumor cells and contaminated cells. It is presently realized that the MHC cosmetics on the surface of those cells is adjusted and the NK cells end up plainly initiated through acknowledgment of "missing self". Typical body cells are not perceived and assaulted by NK cells since they express in place self MHC antigens. Those MHC antigens are perceived by executioner cell immunoglobulin receptors (KIR).

GAMMA/DELTA T CELLS/ΓΔ T CELLS: Like other T cell receptors (TCRs) and Natural Killer T cells, γδ T cells show attributes that place them at the outskirt amongst intrinsic and versatile resistance. On one hand, γδ T cells might be viewed as a segment of versatile invulnerability in that they rework TCR qualities to create junctional decent variety and build up a memory phenotype. In any case, the different subsets may likewise be considered piece of the inborn safe framework where a limited TCR or NK receptors might be utilized as an example acknowledgment receptor.

3.6.2. ADAPTIVE IMMUNITY

The versatile immunity network, otherwise called the gained invulnerable framework or, all the more once in a while, as the particular insusceptible framework, is a subsystem of the general safe framework that is made out of profoundly specific, fundamental cells and procedures that dispose of or counteract pathogen development. The versatile insusceptible framework is one of the two principle insusceptibility systems found in vertebrates. Versatile insusceptibility alludes to antigen-particular invulnerable reaction. The versatile invulnerable reaction is more unpredictable than the inborn. The antigen initially should be prepared and perceived. Once an antigen has been perceived, the versatile safe framework makes a multitude of resistant cells particularly intended to assault that antigen. Versatile invulnerability likewise incorporates a "memory" that makes future reactions against a particular antigen more proficient. Versatile insusceptibility reacts to the test with a high level of specificity and additionally the exceptional property of "memory." Typically, there is a versatile resistant reaction against an antigen inside five or six days after the underlying presentation to that antigen. Introduction to a similar antigen at some point later on brings about a memory reaction: the invulnerable reaction to the second test

happens more rapidly than the to begin with, is more grounded, and is regularly more compelling in killing and clearing the pathogen. The real specialists of versatile insusceptibility are lymphocytes and the antibodies and different atoms they deliver. Versatile framework is predominantly in charge of more mind boggling responses. This framework initiates after inborn reaction is completely actuated. At first, the antigen entered in body is distinguished by the particular insusceptible cells, and afterward a course of responses is begun as antigen immune response to assault the antigen. This resistant framework likewise incorporates creating memory of antigens, which will spare their personalities in the memory cells with the goal that a particular reaction will be started not long after section of a similar pathogen in future. Versatile invulnerability is equipped for perceiving and specifically killing particular remote microorganisms and particles (i.e., outside antigens). Dissimilar to natural resistant reactions, versatile insusceptible reactions are not the same in all individuals from an animal varieties however are response particular to antigenic difficulties. Versatile resistance makes immunological memory after an underlying reaction to a particular pathogen, and prompts an upgraded reaction to ensuing experiences with that pathogen. This procedure of obtained invulnerability is the premise of inoculation. Like the intrinsic framework, the versatile framework incorporates both humoral insusceptibility parts and cell-interceded resistance segments. Gained invulnerability is activated in when a pathogen avoids the inborn safe framework and creates a limit level of antigen and produces "outsider" or "risk" signals enacting dendritic cells. Not at all like the intrinsic invulnerable framework, is the versatile resistant framework very particular to a specific pathogen. Versatile insusceptibility can likewise give dependable insurance; for instance, somebody who recuperates from measles is presently ensured against measles for their lifetime. In obtained resistance, pathogen-particular receptors are "procured" amid the lifetime of the living being. The procured reaction is additionally called as "versatile" on the grounds that it readies the body's insusceptible framework for future difficulties. Obtained resistance is activated when a pathogen dodges the inborn insusceptible framework and creates a limit level of antigen likewise produces "outsider" or "threat" signals enacting dendritic cells.

The significant elements of the procured safe framework include:

- Recognition of particular "non-self" antigens within the sight of "self", amid

the procedure of antigen introduction.

- Generation of reactions that are custom fitted to maximally dispose of particular pathogens or pathogen-contaminated cells.
- Development of immunological memory, in which pathogens are "recollected" through memory B cells and memory T cells.

Adaptive immune responses can be divided into humoral and cell-mediated responses.

HUMORAL IMMUNE RESPONSE: Humoral resistance is that piece of insusceptibility which is intervened by macromolecules found in extracellular liquids, for example, discharged antibodies, supplement proteins, and certain antimicrobial peptides. Humoral insusceptibility is so named in light of the fact that it includes substances found in the humors, or body liquids. Humoral resistance alludes to counter acting agent creation and the extra procedures that go with it, including: Th2 actuation and cytokine generation, germinal focus arrangement and isotype exchanging, proclivity development and memory cell age. It likewise alludes to the effector elements of antibodies, which incorporate pathogen and poison balance, established supplement enactment, and opsonin advancement of phagocytosis and pathogen end. Versatile invulnerability that alludes to antigen-particular segments coursing through the plasma, for example, antibodies, their capacity, and the cells that create them. B cells, sort 2 partner T cells, antibodies, pole cells, and eosinophils are associated with the humoral insusceptible reaction.

Humoral insusceptibility alludes to the part of the versatile resistant reaction that is caused by B cells, antibodies, and sort 2 partner T cells (Th2), and also circling pole cells and eosinophils to a lesser degree. Its name originates from the possibility that blood is one of the humors of the body, since antibodies give latent or dynamic resistance through flow in the circulation system. Sort 2 partner T cells are incorporated into the humoral resistant framework since they exhibit antigens to youthful B-cells, which experience expansion to end up noticeably particular to the displayed antigen. The B cells at that point quickly create an expansive number of antibodies that circle through the body's plasma.

Antibodies give various capacities in humoral insusceptibility. Six unique classes of antibodies give particular capacities and collaborate with various cells in the resistant framework. All antibodies tie to pathogens to opsonize them, which makes it simpler for phagocytic cells to tie to and devastate the pathogen. They likewise kill the poisons delivered by specific pathogens and give supplement pathway actuation, in which flowing proteins are joined in a perplexing course that structures a layer assault complex on a pathogen cell film, which lyses the cell.

Pole cells and eosinophils are considered piece of the humoral insusceptible framework since they can be sharpened towards specific antigens through coursing immunoglobin E (IgE), a particular sort of immunizer delivered by B cells. IgE ties to the pole cells and eosinophils when an antigen is identified, utilizing a sort of Fc receptor on the pole cell or eosinophil that has a high-restricting fondness with IgE. This coupling will cause degranulation and arrival of provocative go betweens that begin an invulnerable reaction against the antigen. This procedure is the motivation behind why memory B cells can cause touchiness (hypersensitivity) development, as circling IgE from those memory cells will actuate a quick provocative and invulnerable respons

CELL-INTERVENED REACTIONS: Adaptive invulnerability that isn't controlled by antibodies and is rather interceded specifically by insusceptible cells themselves, most eminently sort 1 aide T cells and cytotoxic T-cells. The cell of the obtained insusceptible framework is T lymphocytes; T cells are the real sorts of lymphocytes. The human body has around 2 trillion lymphocytes, constituting 20– 40% of white platelets (WBCs). B T cells are gotten from the same multi-strong hematopoietic undifferentiated organisms, and are morphologically vague from each other until after they are actuated, T cells are personally associated with cell-interceded insusceptible reactions. Sort 1 aide T cells and cytoxic T-cells are associated with cell-interceded invulnerable reaction. Cell intervened resistance is controlled by sort 1 partner T cells (Th1) and cytotoxic T cells. These cells are enacted by antigen-exhibiting cells, which make them quickly develop into frames particular to that antigen. White blood cells at that point course through the body to pulverize pathogens in a few ways. Aide T cells encourage the insusceptible reaction by directing cytotoxic T cells to pathogens or

pathogen-tainted cells, which they will then pulverize.

Cytotoxic T cells murder pathogens in a few ways, including the arrival of granules that contain the cytotoxins, perforin and granzyme, which lyse little pores in the layer of a pathogen. At that point T-cell delivered proteases enter the pathogen and actuate an apoptosis reaction inside the cell. Assistant T cells discharge cytokines, for example, interferon-gamma, which can enact cytotoxic T cells and macrophages.

3.7 ACTIVE IMMUNITY

Dynamic immunity alludes to the way toward presenting the body to an antigen to create a versatile invulnerable reaction: the reaction takes days/weeks to grow yet might be durable—even deep rooted. Dynamic invulnerability is the protection created by a person because of an antigenic boost. This includes the dynamic working of the host's safe framework prompting the blend of antibodies and the creation of immunologically dynamic cells. Dynamic invulnerability sets in after an inert period which is required for the immunological hardware to gets under way. Once created dynamic invulnerability is long standing. On the off chance that a person who has been effectively vaccinated against an antigen is presented to same antigen once more, the resistant reactions happen rapidly and plentifully than amid the principal experience. This is known as auxiliary reaction. Dynamic invulnerability is related with immunological memory. This implies the insusceptible framework can hold for long stretches the memory of earlier antigenic introduction. Dynamic insusceptibility gives better insurance then uninvolved resistance. Dynamic resistance can be characteristic or simulated.

Natural dynamic insusceptibility comes about because of either a clinical or an in evident contamination by a microorganism. Such resistance is generally enduring however the length fluctuates with the kind of pathogen. Invulnerability is long lasting after viral illnesses like measles and chickenpox. In flu insusceptibility is fleeting because of antigenic variety, to resistance following the principal disease isn't compelling against second contamination caused by hostile to genically novel infection. In syphilis, an uncommon sort of invulnerability known as 'premunition' is seen. Here, the insusceptibility to re-disease endures just as long as unique contamination stays dynamic.

Artificial dynamic immunity is the protection incited by the immunizations. Antibodies are arrangements of live or executed microorganisms or their items utilized for vaccination.

3.8 PASSIVE RESISTANCE

Passive immunity alludes to the way toward giving IgG antibodies to secure against disease; it gives quick, however fleeting insurance—a little while to 3 or 4 months at most. Detached invulnerability is the protection transmitted inactively to a person in a 'readymade' shape. There is no antigenic jolt; rather, preformed antibodies are directed. There is no inactive period, assurance being taking effect right now. The invulnerability is transient, no optional reaction in aloof resistance. It is less successful then dynamic vaccination. The fundamental favorable position of uninvolved vaccination is that it demonstrations promptly and, along these lines, can be utilized when quick impact is wanted, for instance against diphtheritic serum given to a youngster giving diphtheria.

Natural detached immunity is the protection latently exchanged from mother to infant. In the human newborn children, maternal antibodies are transmitted dominatingly through the placenta. It is just by the age of three months that the newborn child obtains some measure of immunological freedom.

Artificial detached immunity is the protection inactively exchanged to a beneficiary by the organization of antibodies. The specialists utilized for this reason for existing are hyperimmune sera of creature or human starting point (Anti lockjaw serum, ATS, arranged from hyperimmune steeds) and pooled human gamma-globulin (lockjaw resistant globulin, TIG).

Some of the time a blend of dynamic and inactive inoculation is utilized, known as consolidated vaccination. For instance, security of a nonimmune individual with a lockjaw inclined injury (both TIG and Tetanus toxoid is given).

3.9 SUMMARY

Immunity is the condition of insurance against outside living beings or substances

(antigens). Vertebrates have two sorts of invulnerability, inborn and versatile. Intrinsic and versatile resistance work in agreeable and reliant ways. The enactment of inborn insusceptible reactions produces flags that animate and direct consequent versatile safe reactions. Intrinsic resistance isn't particular to any one pathogen yet rather constitutes a first line of guard, which incorporates anatomic, physiologic, endocytic and phagocytic, and provocative boundaries. Versatile safe reactions display four immunologic characteristics: specificity, assorted variety, memory, and self/non-self acknowledgment. The high level of specificity in versatile resistance emerges from the exercises of particles (antibodies and T-cell receptors) that perceive and tie particular antigens. The insusceptible framework produces both humoral and cell-interceded reactions. The humoral reaction is most appropriate for end of exogenous antigens; the cell-intervened reaction, for end of endogenous antigens. Procured invulnerability is the protection that an individual secures amid life. Gained invulnerability is of two sorts: Active insusceptibility is the protection created by a person because of an antigenic jolt. Latent insusceptibility is the protection transmitted inactively to a person in a 'readymade' shape. Both can be subdivided into common and manufactured.

3.10 GLOSSARY

Adjuvant: Any substance which nonspecifically enhances the immune response to antigen.

Allergen: An antigen which causes allergy.

Allergy: IgE-mediated hypersensitivity.

Antigen: Any molecule capable of being recognized by an antibody or T-cell receptor

Apoptosis: A form of programmed cell death, characterized by endonuclease digestion of DNA.

Basophil: A type of granulocyte found in the blood and resembling the tissue mast cell.

Cell-mediated immunity (CMI): Refers to T-cell mediated immune responses.

Chemotaxis: Movement of cells up a concentration gradient of chemotactic factors.

Complement: A group of serum proteins, some of which act in an enzymatic cascade, producing effector molecules involved in inflammatio, phagocytosis and cell

lysis.

Cytokines: Low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells.

Cytotoxic: Kills cells.

Edema: Swelling caused by accumulation of fluid in the tissues.

Eosinophil: A class of granulocyte, the granules of which contain toxic cationic proteins.

Erythema: The redness produced during inflammation due to erythrocytes entering tissue spaces.

Exudate: The extravascular fluid (containing proteins and cellular debris) which accumulates during inflammation.

Fibroblast: Connective tissue cell which produces collagen and plays an important part in wound healing.

Granulocyte: Myeloid cells containing cytoplasmic granules i.e. neutrophils, eosinophils and basophils.

Humoral: Pertaining to extracellular fluid such as plasma and lymph. The term humoral immunity is used to denote antibody-mediated immune responses.

Immunogen: Any substance which elicits an immune response.

Inflammation: The tissue response to trauma, characterized by increased blood flow and entry of leukocytes into the tissues, resulting in swelling, redness, elevated temperature and pain.

Innate immunity: Immunity which is not intrinsically affected by prior contact with antigen, i.e. all aspects of immunity not directly mediated by lymphocytes.

Kuppfer cells: Fixed tissue macrophages lining the blood sinuses in the liver.

Leukocyte: White blood cells, which include neutrophils, basophils, eosinophils, lymphocytes and monocytes.

Lymph: The tissue fluid which drains into and through the lymphatic system.

Macrophage: Large phagocytic cell, derived from the blood monocyte.

Mast cell: A tissue cell with abundant granules which resembles the blood basophil

Opsonin: Substance, which enhances phagocytosis by promoting adhesion of the antigen to the phagocyte.

Opsonization: Coating of antigen with opsonin to enhance phagocytosis

Phagocyte: Cells, including monocytes/macrophages and neutrophils, which are

specialized for the engulfment of cellular and particulate matter.

Primary immune response: The relatively weak immune response which occurs upon the first encounter of naive lymphocytes with a given antigen

Secondary immune response: The qualitatively and quantitatively improved immune response which occurs upon the second encounter of primed lymphocytes with a given antigen

3.11 SELF ASSESSMENT QUESTION AND POSSIBLE ANSWERS

Multiple Choice Questions:

(1) Which of the following does **not** protect body surfaces:

(a) Skin (b) Mucus

(c) Gastric acid (d) Salivary amylase

2) Acute inflammation characteristically involves:

(a) Constriction of arterioles. (b) Capillary endothelial cell enlargement

(c) Influx of macrophages (d) Influx of neutrophils

3) Lysozyme:

(a) Is a cytoplasmic organelle (b) Activates complement

(c) Is a proteolytic enzyme (d) Splits peptidoglycan

4) Interferons:

(a) Are found only in mammalian species (b) Are divided into 5 main families

(c) Induce enzyme synthesis in the target cell (d) Only affect infected cells

5) Natural killer (NK) cells do not:

(a) Respond to interferon (b) Contain perforin

(c) Contain tumor necrosis factor (TNF). (d) Kill only by damaging the target cell outer membrane

Answers:

1) Salivary amylase 2) Influx of neutrophils
3) Splits peptidoglycan 4) Induce enzyme synthesis in the target cell.
5) Kill only by damaging the target cell outer membrane

Fill in the blanks:

1. Humoral immunity is mediated by antibodies from _____.

2. Cell mediated immunity is mediated by _____s.

3. Adaptive immune system provides _____ & _____.

4. _____ are low molecular weight cytokines important in inflammation.

5. _____ are not part of innate immune response.

Answer:

1. B lymphocytes. 2. T lymphocytes.

3. Specificity & memory.4.Chemokines

5. B cells

Short Answer Type Questions:

. 1. Maternal antibodies transferred to foetus through placenta provide which type of immunity?

Ans. Natural passive immunity

2. Name phagocytic cells?

Ans. Neutrophils, Macrophages & Histiocytes.

3. Define Natural killer cells in one sentence?

Ans. The cells which are able to kill virus-infected cells without prior sensitization.

4. What are the three main functions of complement system?

Ans. Promote inflammation, opsonisation & cell lysis.

5. A primary immune response in an adult human requires approximately how much time to produce detectable antibody levels in the blood?

Ans. It requires one week time to produce detectable antibody levels in blood.

Long Answers Type Questions

1. What are the differences between active and passive immunity? Describe with examples?
2. Explain innate immunity & its types with examples?
3. What is humoral immunity explain?
4. Describe working of Cell mediated immunity with suitable examples?
5.Explain adaptive immunity & its types with examples?

3.12 REFERENCES

Kind, Thomas J., Goldsby, Richard A., Osborne, Barbara Anne. and Kuby, Janis. (2007). Kuby Immunology. New York: W.H. Freeman

Lydyard,P.M., Whelan, A. and Fanger, M.W. (2004). Immunology. Garland Science/BIOS Scientific Publishers Limited.

Stvrtinova, Viera, Jan Jakubovsky, Ivan Hulin (1995). Inflammation and Fever from Pathophysiology: Principles of Disease. Computing Centre, Slovak Academy of Sciences: Academic Electronic Press.

UNIT:4 CELLS &ORGANS OF THE IMMUNE SYSTEM

CONTENTS

4.1 OBJECTIVES

- To list the types of cells & organs of the immune system
- To describe the concepts of primary lymphoid organs
- To explain the mechanism of lymphatic system
- To explain the secondary lymphoid organs

4.2INTRODUCTION

The immune system is a host resistance framework involving numerous natural structures and procedures inside a life form that ensures against malady. To work legitimately, an insusceptible framework must identify a wide assortment of specialists, known as pathogens, infections, parasitic worms, and recognize them from the creature's own sound tissue. The insusceptible framework comprises of a wide range of organs and tissues that are situated all through the body. They are called lymphoid organs sice they are home to lymphocytes, little white platelets that are the key players in the insusceptible framework and convey by means of the circulatory frameworks (blood and lymph). The safeguard arrangement of the human body is comprised of whole organs and vessel frameworks like the lymph vessels, yet additionally of individual cells and proteins.

The lymphatic framework is a piece of the circulatory framework, containing a system of conductors called lymphatic vessels that convey reasonable liquid, called lymph, unidirectional towards the heart. The lymphatic framework has different interrelated capacities including the transportation of white platelets to and from the lymph hubs into the bones, and the transportation of antigen-introducing cells, (for example, dendritic cells) to the lymph hubs where an invulnerable reaction is invigorated. Lymphoid tissue is found in numerous organs, especially the lymph hubs. A liquid called lymph, (lymph = clear liquid) streams in lymphatic vessels, lymphatic tissue and red bone marrow. Liquid sift through of vessels and channels into lymphatic vessels to wind up lymph. The substance of lymph is the same as interstitial liquid, the liquid around tissue cells. Lymph in the long run depletes into

venous blood. Lymph channels interstitial liquid, transports dietary lipids and encourages insusceptible reactions. Lymphoid organs shape some portion of the body's resistant framework. As blood circles under weight, its liquid part (plasma) leaks through the thin mass of the vessels into the encompassing tissue. A lot of this liquid, called interstitial liquid, comes back to the blood through the fine layers. The rest of the interstitial liquid, now called lymph, streams from the spaces in connective tissue into a system of modest open lymphatic vessels and afterward into a progression of dynamically bigger gathering vessels called lymphatic vessels.

Lymphoid organs safeguard the body against attacking pathogens that reason diseases or spread of tumors. These organs exist as essential, auxiliary or tertiary, in view of the phase of lymphocyte improvement and development the organ is associated with. These organs comprise of connective tissues with various sorts of leukocytes or white platelets. Lymphocytes typically show the most elevated rate among these white platelets paying little heed to the sort of lymphoid organ (i.e. essential, optional or tertiary).

Various morphologically and practically different organs and tissues have different capacities in the improvement of safe reactions. These can be recognized by work as the essential and optional lymphoid organs .The thymus and bone marrow are the essential lymphoid organs, where development of lymphocytes happens. The lymph hubs, spleen, and different mucosal related lymphoid tissues (MALT, for example, gut-related lymphoid tissue (GALT) are the auxiliary (or fringe) lymphoid organs, which trap antigen and give destinations to develop lymphocytes to cooperate with that antigen. Moreover, tertiary lymphoid tissues, which ordinarily contain less lymphoid cells than optional lymphoid organs.

4.3 ORGANS OF IMMUNE SYSTEM

4.3.1 PRIMARY LYMPHOID ORGANS

The essential lymphoid organs produce lymphocytes from youthful forebear cells. There are two essential lymphatic organs, the red bone marrow and the thymus organ,

where development of lymphocytes happens.

1)Bone marrow: Bone marrow is the light and adaptable tissue inside in the inside of bones, including the hip and thigh bones. Bone marrow contains juvenile cells, called undifferentiated organisms. In people, red platelets are created by centers of bone marrow in the heads of long bones in a procedure known as hematopoiesis. The hematopoietic part of bone marrow creates roughly 500 billion platelets for every day and it is additionally a key segment of the lymphatic framework, delivering the lymphocytes that help the body's insusceptible framework. The two sorts of bone marrow are "red marrow" (Latin: medulla ossium rubra), which comprises predominantly of hematopoietic tissue, and "yellow marrow" (Latin: medulla ossium flava), which is for the most part comprised of fat cells. Red platelets, platelets, and most white platelets emerge in red marrow. The two sorts of bone marrow contain various veins and vessels. During childbirth, all bone marrow is red. With age, increasingly of it is changed over to the yellow sort; just around half of grown-up bone marrow is red. Red marrow is discovered primarily in the level bones, for example, the pelvis, sternum, noggin, ribs, vertebrae and scapulae, and in the cancellous ("elastic") material at the epiphyseal closures of long bones, for example, the femur and humerus. Yellow marrow is found in the medullary pit, the empty inside of the center segment of short bones. In instances of serious blood misfortune, the body can change over yellow marrow back to red marrow to build platelet generation. The bone marrow contains hematopoietic foundational microorganisms, which offer ascent to the three classes of platelets that are found in the course: white platelets (leukocytes), red platelets (erythrocytes), and platelets (thrombocytes). Both T-cell and B-cells are 'conceived' in the bone marrow. In any case, while B cells additionally develop in the bone marrow, T-cells need to move to the thymus, which is the place they develop in the thymus.

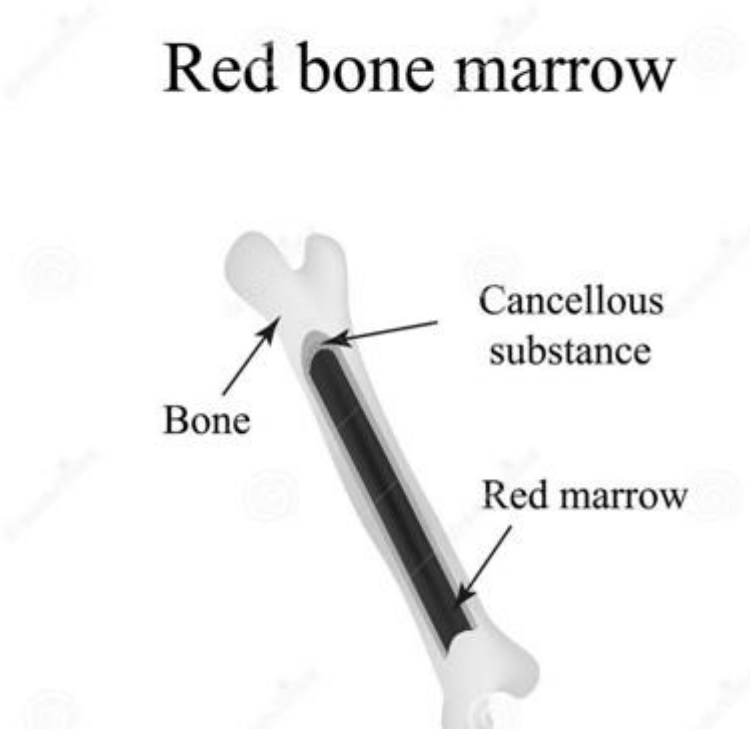


Fig.4.1 Red bone marrow

2) B Lymphocytes: B cells, otherwise called B lymphocytes, are a sort of white platelet of the lymphocyte subtype; B cells develop in the bone marrow, which is at the center of generally bones. In feathered creatures, B cells develop in the **Bursa of Fabricius**, a lymphoid organ thus named B lymphocytes. They work in the humoral invulnerability part of the versatile safe framework by emitting antibodies. B cells display antigen i.e. antigen-introducing cells (APCs) and emit cytokines. B cells, not at all like the other two classes of lymphocytes, T cells and common executioner cells, express B cell receptors (BCRs) on their cell film. BCRs enable the B cell to tie a particular antigen, against which it will start a counter acting agent reaction. B cells create from hematopoietic undifferentiated organisms (HSCs) that begin from bone marrow. HSCs first separate into multipotent forebear (MPP) cells, at that point normal lymphoid begetter (CLP) cells. From here, their advancement into B cells happens in a few phases.

For finish advancement, youthful B cells relocate from the bone marrow to the spleen and in addition go through two transitional stages: T1 and T2. All through their relocation to the spleen and after spleen section, they are viewed as T1 B cell. Once separated, they are called as develop B cells, or gullible B cells. They

have an exceptional antigen restricting site or receptor mIGM (layer immuno globulin). B cell comprise of two indistinguishable overwhelming and light fastened polypeptide which are connected by disulphide bond. The carboxylic terminal end of both light and substantial chain included a separated inside which antigen ties. B cell initiation happens in the optional lymphoid organs (SLOs, for example, the spleen and lymph hubs. After B cells develop in the bone marrow, they relocate through the blood to SLOs, which get a steady supply of antigen through flowing lymph. At the SLO, B cell actuation starts when the B cell ties to an antigen through its BCR. In the wake of communicating with the antigen it separate into fleeting plasmablasts for quick insurance and extensive plasma cells and memory B cells for relentless assurance.

3) B cell types: B cells are of following types depending on the size & nature of work they perform.

4)Plasma cell: An extensive, non-multiplying counter acting agent emitting cell emerging from B cell separation. There B cells initially separate into a plasma-impact like cell, at that point separate into a plasma cell. Plasma cells are created later in a disease and, contrasted with plasmablasts, have antibodies with a higher liking towards their objective antigen because of proclivity development in the germinal focus (GC) and deliver more antibodies. Plasma cells regularly result from the germinal focus response from T cell-subordinate initiation of B cells, be that as it may they can likewise come about because of T cell-autonomous enactment of B cells. Neutralizer discharging plasma cells free receptors and develop into extensive size with huge number of E.R. and begin decreasing antibodies which are connected in free course. Plasma cells are called manufacturing plants creating substantial number of antibodies; they can discharge as much as 2000 antibodies particles for every second and life expectancy is just for few days.

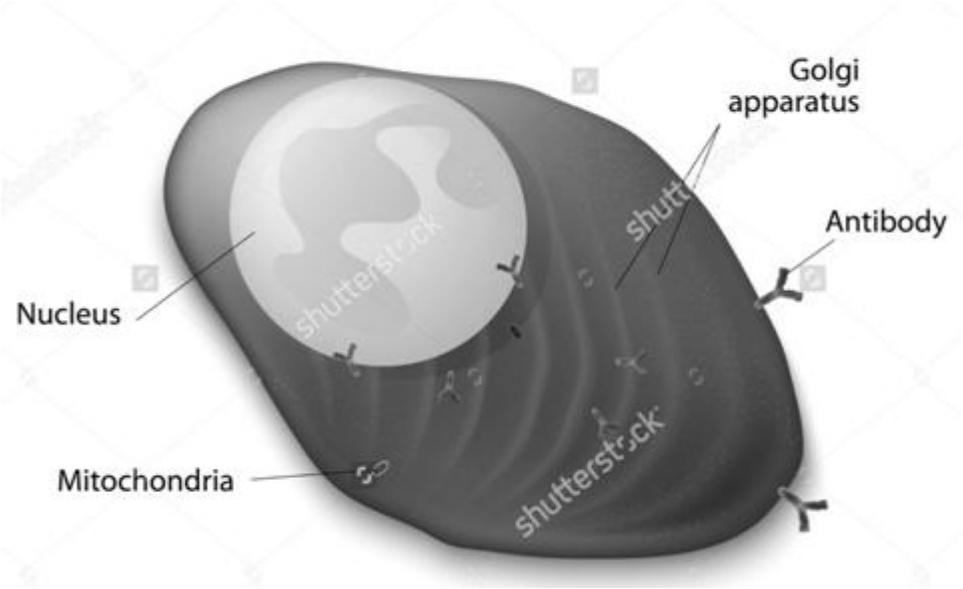


Fig 4.2Structure of plasma Cell

5)Memory B cell: Lethargic B cell emerging from B cell separation. Their capacity is to circle through the body and start a more grounded, more quick neutralizer reaction (known as the optional immune response reaction) in the event that they recognize the antigen that had initiated their parent B cell (memory B cells and their parent B cells share the same BCR, subsequently they distinguish a similar antigen). Memory B cells can be produced from T cell-subordinate actuation through both the additional follicular reaction and the germinal focus response and from T cell-free initiation of B1 cells. Memory cells just deliver immune response for articulation on their cell surface, there life traverse is relatively long to plasma cells.

6) Plasmablast:A brief, multiplying counter acting agent discharging cell emerging from B cell separation. Plasmablasts are created right on time in a disease and their antibodies have a tendency to have a weaker proclivity towards their objective antigen contrasted with plasma cell. Plasmablasts can come about because of T cell-free enactment of B cells or the extrafollicular reaction from T cell-subordinate actuation of B cells.

7) Follicular (FO) B cell: Otherwise called a B-2 cell, most normal sort of B cell and, when not circling through the blood, is discovered primarily in the lymphoid follicles of optional lymphoid organs (SLOs). They are in charge of creating the lion's share of high-proclivity antibodies amid a disease.

8) **Marginal zone (MZ) B cell:** Discovered essentially in the negligible zone of the spleen and fills in as a first line of resistance against blood-borne pathogens, as the peripheral zone gets a lot of blood from the general dissemination. They can experience both T cell-autonomous and T cell-subordinate actuation, however specially experience T cell-free enactment.

9)B-1 cell: Arises from a developmental pathway different from FO B cells and MZ B cells.

10) Regulatory B (Breg) cell: An immunosuppressive B cell sort that stops the extension of pathogenic, master fiery lymphocytes through the discharge of IL-10, IL-35, and TGF-β. Additionally, it advances the age of administrative T (Treg) cells by straightforwardly communicating with T cells to skew their separation towards Tregs. All B cell sorts can separate into a Breg cell through instruments including fiery signs and BCR acknowledgment.

11)Thymus gland: The thymus is a particular essential lymphoid organ of the invulnerable framework, it is found anatomically in the foremost unrivaled mediastinum, before the heart and behind the sternum. The thymus is a pinkish-dark shading, delicate, and lobulated structure, during childbirth it is around 5 cm long, 4 cm in broadness, and around 6 mm in thickness. The organ grows amid adolescence, and decays at pubescence and in grown-ups it is yellow. It is inside zonated into numerous lobules which are isolated from each other by connective tissue strand called Trabecullae. Every lobule comprise of focal medulla and external cortex. Inside the thymus, T cells or T lymphocytes develop, the cortex and medulla assume distinctive parts in the advancement of T-cells. The thymus gives an inductive domain to improvement of T cells from hematopoietic ancestor cell, it is biggest and most dynamic amid the neonatal and pre-immature periods. The cortex is the area of the most punctual occasions in thymocyte improvement and is chiefly made out of lymphocytes. In medulla partition, the lymphoid cells are generally less in number and the area of the last occasions in thymocyte improvement. The two fundamental segments of the thymus, the lymphoid thymocytes and the thymic epithelial cells, have particular formative inceptions. The two fundamental segments of the thymus, the lymphoid thymocytes and the thymic epithelial cells, have particular formative

inceptions. The begetter T cells enter the thymus and begin multiplying inside the cortex, which is combined with Apoptosis, at that point a little extent of surviving T cells, relocate to medulla where they keep on maturing and at last leave the thymus. Thymus is made out of system of stromal cells, epithelial cells, interdigitating dendritic cells and macrophages which contribute for the development of thymocytes.

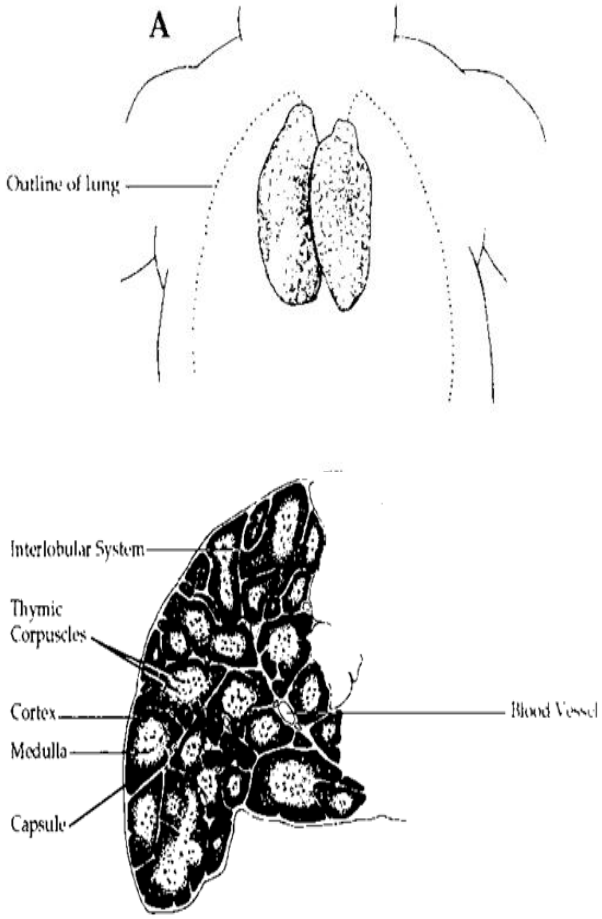


Fig 4.3 Structure of Thymus Gland

12) T Lymphocytes: T lymphocytes infer their name since they develop in the thymus from thymocytes. A T cell, or T lymphocyte, is a kind of lymphocyte (a subtype of white platelet) that assumes a focal part in cell-interceded invulnerability. Immune system microorganisms can be recognized from different lymphocytes, for example, B cells and common executioner cells, by the nearness of a T-cell receptor on the cell surface. Immune system microorganisms can be recognized from different lymphocytes, for example, B cells and regular executioner cells, by the nearness of a T-cell receptor on the cell surface. All T cells begin from haematopoietic

undifferentiated organisms in the bone marrow. Haematopoietic begetters (lymphoid forebear cells) from haematopoietic undifferentiated cells populate the thymus and extend by cell division to produce a substantial populace of juvenile thymocytes. A one of a kind component of T cells is their capacity to separate amongst solid and unusual (e.g. contaminated or harmful) cells in the body. Like B lymphocytes, these cells have layer receptors for antigen. In spite of the fact that the antigen restricting T-cell receptor is basically unmistakable from immunoglobulin, it shares some regular basic highlights with the immunoglobulin particle, most prominently in the structure of its antigen-restricting site. Not at all like the layer bound immune response on B cells, however, the T-cell receptor (TCR) does not perceive free antigen. Rather the TCR perceives just antigen that is bound to specific classes of self-particles. Most T cells perceive antigen just when it is bound to a self-particle encoded by qualities inside the major histo-similarity complex (MHC). The T cell is confined to restricting antigen showed on self-cells just however to be perceived by most T cells, this antigen must be shown together with MHC particles on the surface of antigen-displaying cells or on infection contaminated cells, tumor cells, and unions. The T-cell framework has created to dispense with these modified self-cells, which represent a danger to the ordinary working of the body. Like B cells, T cells express unmistakable film atoms. All T-cell subpopulations express the T-cell receptor, a complex of polypeptides that incorporates CD3; and most can be recognized by the nearness of either of two film particles, CD4 and CD8. The capacity of T cells to overlook solid cells yet react when these same cells contain pathogen (or tumor) determined pMHC is known as antigen segregation.

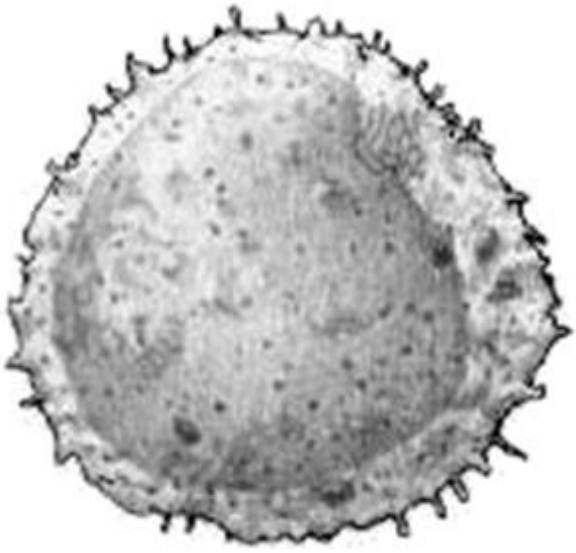


Fig 4.4 Structure of Lymphocytes

13) T cell types: The types of T cell is as mentioned below:

a. Effector cells: The classification of effector T cell is an expansive one that incorporates different T cell sorts that effectively react to a jolt, for example, co-incitement. This incorporates partner, executioner, administrative, and conceivably other T cell sorts.

b. Helper cells (T_H cells):T-aide cells help other white platelets in immunologic procedures, including development of B cells into plasma cells and memory B cells, and initiation of cytotoxic T cells and macrophages. These cells are otherwise called CD4+ T cells since they express the CD4 glycoprotein on their surfaces. Aide T cells end up plainly actuated when they are given peptide antigens by MHC class II atoms, which are communicated on the surface of antigen-exhibiting cells (APCs). Once initiated, they isolate quickly and discharge little proteins called cytokines that control or aid the dynamic insusceptible reaction. These cells can separate into one of a few subtypes, including TH1, TH2, TH3, TH17, TH9, or TFH, which discharge distinctive cytokines to encourage diverse sorts of insusceptible reactions. Motioning from the APC coordinates T cells into specific subtypes.

c. Cytotoxic/killer cells (T_C cells): Cytotoxic T cells otherwise called TC cells, CTLs, T-executioner cells, executioner T cells devastate infection contaminated cells and tumor cells, and are additionally ensnared in transplant dismissal. These cells are

otherwise called CD8+ T cells since they express the CD8 glycoprotein at their surfaces. These cells perceive their objectives by authoritative to antigen related with MHC class I particles, which are available on the surface of every single nucleated cell. Through IL-10, adenosine, and different atoms emitted by administrative T cells, the CD8+ cells can be inactivated to an anergic state, which avoids immune system illnesses.

d. Memory cells: Memory T cells are a subset of antigen-particular T cells that persevere long haul after a disease has settled. They rapidly extend to substantial quantities of effector T cells upon re-presentation to their related antigen, therefore furnishing the insusceptible framework with "memory" against past diseases. Memory cells might be either CD4+ or CD8+. Memory T cells ordinarily express the cell surface protein CD45RO. Memory T cells involve three subtypes; focal memory T cells (TCM cells), effector memory T cells (TEM cells and TEMRA cells) and occupant memory T cells (TRM).

e. Natural killer T cell: Natural killer T cells (NKT cells) ought not to be mistaken for common killer cells of the intrinsic immune system which connect the versatile immune network with the natural insusceptible framework. Dissimilar to regular T cells that perceive peptide antigens displayed by major histo-similarity complex (MHC) atoms, NKT cells perceive glycol-lipid antigen exhibited by a particle called CD1d. Once enacted, these cells can perform capacities credited to both Th and Tc cells (i.e., cytokine creation and arrival of cytolytic/cell murdering atoms). They are likewise ready to perceive and take out some tumor cells and cells tainted with herpes infections.

f. Gamma delta T cells: Gamma delta T cells (γδ T cells) speak to a little subset of T cells that have a particular T cell receptor (TCR) on their surfaces. A dominant part of T cells have a TCR made out of two glycoprotein chains called α-and β-TCR chains. Notwithstanding, in γδ T cells, the TCR is comprised of one γ-chain and one δ-chain. This gathering of T cells is significantly less regular in people (around 2% of aggregate T cells); and are discovered generally in the gut mucosa, inside a populace of lymphocytes known as intraepithelial lymphocytes. Be that as it may, γδ T cells are not MHC-confined and appear to have the capacity to perceive entire proteins instead

of expecting peptides to be introduced by MHC particles on APCs. Some murine γδ T cells perceive MHC class IB particles, however. Human Vγ9/Vδ2 T cells, which constitute the major γδ T cell populace in fringe blood, are exceptional in that they particularly and quickly react to an arrangement of nonpeptidic phosphorylated isoprenoid antecedents, all things considered named phosphoantigens, which are delivered by for all intents and purposes every living cell.

5.3.2 SECONDARY LYMPHOID ORGANS

Optional or fringe lymphoid organs, which incorporate lymph hubs and the spleen, keep up develop credulous lymphocytes and start a versatile invulnerable reaction. The fringe lymphoid organs are the locales of lymphocyte actuation by antigens. Actuation prompts clonal extension and partiality development. Develop lymphocytes re-flow between the blood and the fringe lymphoid organs until the point that they experience their particular antigen. Auxiliary lymphoid tissue gives nature to the remote or adjusted local particles (antigens) to connect with the lymphocytes. Auxiliary lymphoid tissues are orchestrated as a progression of channels checking the substance of the extracellular liquids, i.e. lymph, tissue liquid and blood. The lymphoid tissue sifting each of these liquids is organized in various ways. Optional lymphoid tissues are additionally where lymphocytes are actuated. These include: lymph hubs, tonsils, spleen, Peyer's patches and mucosa related lymphoid tissue (MALT).

1. Lymph nodes: A lymph hub is an oval-or kidney-formed organ of the lymphatic framework, exhibit broadly all through the body including the armpit and stomach and connected by lymphatic vessels. Lymph hubs are real destinations of B, T, and other invulnerable cells. Lymph hubs are imperative for the best possible working of the resistant framework, going about as channels for outside particles and disease cells. A lymph hub is a sorted out accumulation of lymphoid tissue, through which the lymph passes on its way back to the blood. Lymph hubs are situated at interims along the lymphatic framework. A few afferent lymph vessels get lymph, which permeates through the substance of the lymph hub, and is then depleted out by an efferent lymph vessel. There are in the vicinity of five and six hundred lymph hubs in the human body, huge numbers of which are gathered in bunches in various districts

as in the underarm and stomach regions. Lymph hub groups are generally found at the base of appendages (crotch, armpits) and in the neck, where lymph is gathered from areas of the body liable to support pathogen tainting from wounds. The substance of a lymph hub comprises of lymphoid follicles in an external segment called the cortex. The inward part of the hub is known as the medulla, which is encompassed by the cortex on all sides with the exception of a bit known as the hilum. The hilum introduces as a misery on the surface of the lymph hub, making the generally round lymph hub be bean-molded or ovoid. The efferent lymph vessel straightforwardly rises up out of the lymph hub at the hilum. The conduits and veins providing the lymph hub with blood enter and exit through the hilum. The district of the lymph hub called the para-cortex quickly encompasses the medulla. Not at all like the cortex, which has generally youthful T cells, or thymocytes, the paracortex has a blend of juvenile and develop T cells. Lymphocytes enter the lymph hubs through specific high endothelial venules found in the paracortex. A lymph follicle is a thick gathering of lymphocytes, the number, size and setup of which change as per the utilitarian condition of the lymph hub. For instance, the follicles extend fundamentally while experiencing an outside antigen. The choice of B cells, or B lymphocytes, happens in the germinal focus of the lymph hubs. Lymph hubs are especially various in the mediastinum in the chest, neck, pelvis, axilla, inguinal locale, and in relationship with the veins of the digestion tracts.

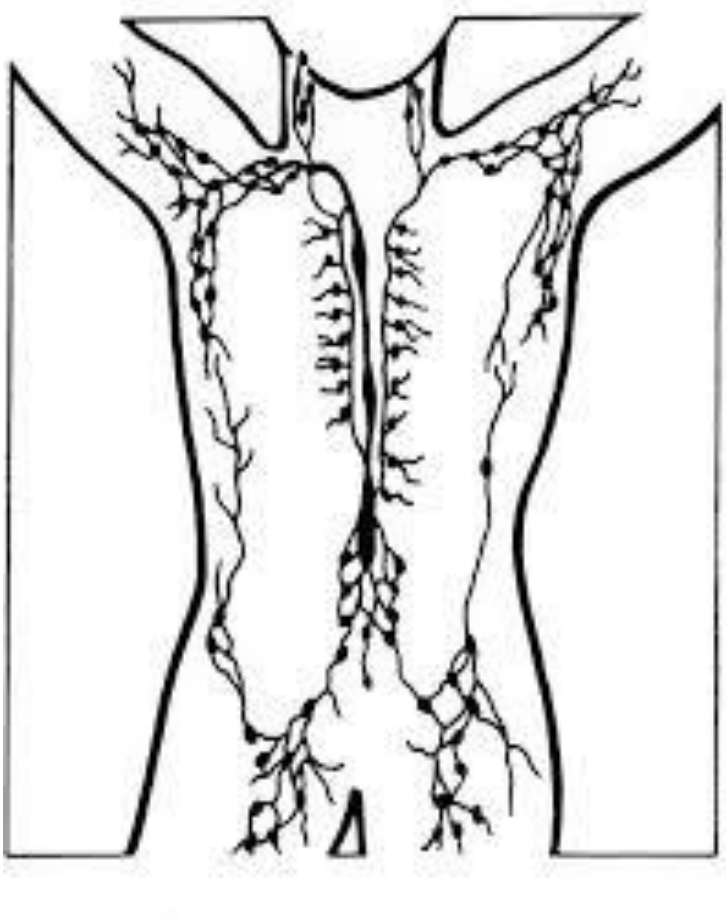


Fig 4.5 Secondry lymphoid organs

Function: The lymph liquid inside the lymph hubs contains lymphocytes, a kind of white platelet, which consistently re-circles through the lymph hubs and the circulation system. Atoms found on microscopic organisms cell dividers or concoction substances discharged from microorganisms, called antigens, might be taken up by devoted antigen-displaying cells, for example, dendritic cells into the lymph framework and afterward into lymph hubs. In light of the antigens, the lymphocytes in the lymph hub make antibodies which will leave the lymph hub into flow, look for, and focus on the pathogens creating the antigens by focusing on them for devastation by different cells. On the off chance that the lymphocytes can't battle a specific pathogen, the general invulnerable framework will be initiated to help. The expanded quantities of resistant framework cells battling the contamination will influence the hub to extend and end up plainly swollen. .They end up plainly aroused or amplified in different contaminations and sicknesses which may go from throat

diseases, to perilous growths. The state of the lymph hubs is essential in tumor arranging, which chooses the treatment to be utilized, and decides the anticipation. Whenever swollen, excited or developed, lymph hubs can be hard, firm or delicate. The lymph is sifted by lymph hubs, which are cases of epitomized lymphoid tissue. There are around 100-200 of these which generally happen in the neck, thorax, guts and pelvis. They contain B-and T-cells, which for the most part enter the hubs through the circulatory system, and furthermore contain macrophages.

2. Spleen: The spleen is an organ found in all vertebrates. Comparable in structure to an extensive lymph hub, it acts basically as a blood channel. It is a substantial, ovoid optional lymphoid organ arranged high in the left stomach cavity. It is a substantial embodied organ; with external case is fibro-versatile. The diaphragmatic surface of the spleen or phrenic surface is raised, smooth, and is coordinated upward, in reverse, and to one side, with the exception of at its upper end, where it is coordinated marginally to the center. The spleen lies underneath the left stomach, underneath the ninth, tenth, and eleventh ribs. The stomach isolates the spleen from the pleura and base of the left lung. The instinctive surface of the spleen is isolated by an edge into two areas: a foremost or gastric and a back or renal. The gastric surface is coordinated forward, upward, and toward the center, is wide and curved, and is in contact with the back mass of the stomach. Underneath this it is in contact with the tail of the pancreas. The spleen assumes imperative parts as to red platelets (additionally alluded to as erythrocytes) and the safe framework. It expels old red platelets and holds a save of blood, which can be important if there should arise an occurrence of hemorrhagic stun, and furthermore reuses press. As a piece of the mononuclear phagocyte framework, it uses hemoglobin expelled from senescent erythrocytes. The spleen integrates antibodies in its white mash and evacuates counter acting agent covered microbes and immune response covered platelets by method for blood and lymph hub flow. The red mash of the spleen shapes a repository that contains over portion of the body's monocytes. These monocytes, after moving to harmed tissue, (for example, the heart after myocardial dead tissue), transform into dendritic cells and macrophages while advancing tissue recuperating. The spleen is a focal point of action of the mononuclear phagocyte framework and can be viewed as undifferentiated from a substantial lymph hub. Spleen reaction to orderly contamination since it channels the

blood and traps the blood borne antigens. Inside to spleen is compartmentalized structure which is isolated by connective tissue projection called Tribeculate. These compartments are of two sorts; red mash which is rich in RBCs and macrophages and white mash which encompasses the corridors framing pariartrial lymphatic sheath i.e. Buddies and for the most part comprise of T lymphocytes. The negligible zone situated by the PALS is comprised of B cells and macrophages sorted out into lymphoid follicle. Blood going through the spleen will convey antigen and discharge it in the minor zone, in the wake of entering the minimal zone they are caught by dendritic cells which convey the antigen to the PALS.

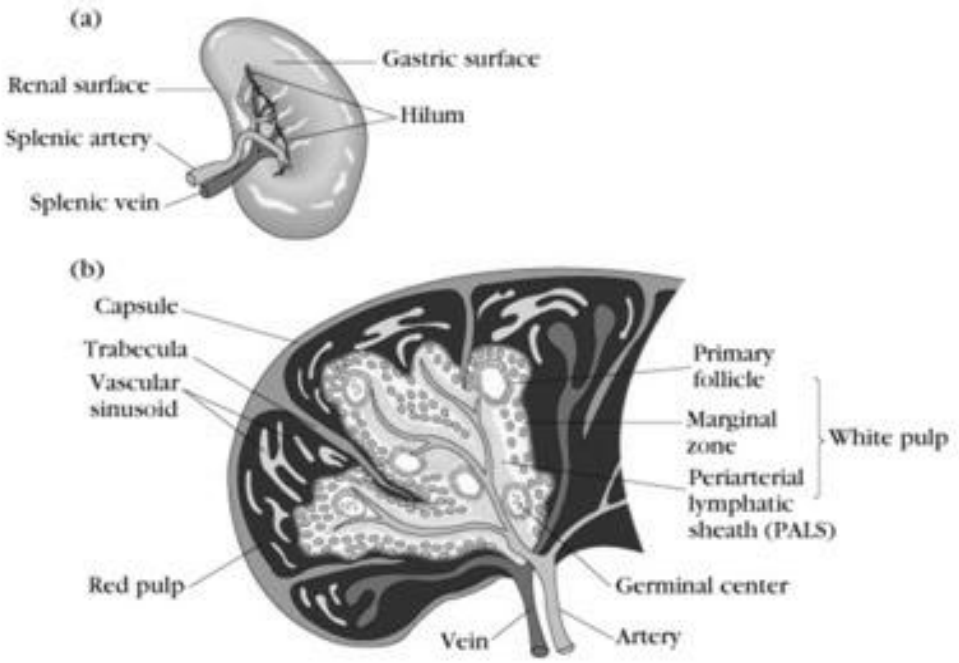


Fig 4.6

MALT(mucosal associated lymphoid tissues): The mucosa-related lymphoid tissue (MALT), additionally called mucosa-related lymphatic tissue, is a diffuse arrangement of little groupings of lymphoid tissue found in different sub-mucosa layer destinations of the body, for example, the gastrointestinal tract, thyroid, bosom, lung, salivary organs, eye, and skin. Larger part of lymphoid tissue in human body is situated inside the mucosal coating of the stomach related, respiratory, and urogenital tract; this is on the grounds that these are principle destinations of passage of antigens. The guard at the surface of mucosal covering is given by a gathering of

lymphoid tissues by and large known as MALT. MALT is populated by lymphocytes, for example, T cells and B cells, and in addition plasma cells and macrophages, each of which is all around arranged to experience antigens going through the mucosal epithelium. On account of intestinal MALT, M cells are likewise present, which gather antigen from the lumen and convey it to the lymphoid tissue. Fundamentally, these tissues extend from free, scarcely composed bunches of lymphoid cells in the lamina propria of intestinal villi to efficient structures, for example, the recognizable tonsils and reference section, and additionally Peyer's patches, which are found inside the sub-mucosal layer of the intestinal coating. The practical significance of MALT in the body's guard is by its substantial populace of counter acting agent creating plasma cells, whose number far surpasses that of plasma cells in the spleen, lymph hubs, and bone marrow consolidated.

3. Tonsils: Tonsils are the two lymph hubs situated on each side of the back of your throat. These are accumulations of lymphoid tissue looking into the air stomach related tract. Tonsils are extensive somewhat epitomized masses of lymphoid tissue, found in the dividers of the pharynx and naso-pharynx, and at the base of the tongue. They frame a fragmented ring around the gastrointestinal and respiratory tracts, where they traverse .They work as a protection component. They help keep our body from disease. At the point when the tonsils wind up plainly tainted, the condition is called tonsillitis. Tonsils in people incorporate, from foremost (front), unrivaled (top), (back), and substandard (base) i.e. the adenoid tonsil, two tubal tonsils, two palatine tonsils, and the lingual tonsil individually. Tonsils tend to achieve their biggest size close pubescence, and they step by step experience degeneration from that point. These tissues are the invulnerable framework's initially line of resistance against ingested or breathed in outside pathogens. Tonsils have on their surface specific antigen catch cells called M cells that consider the take-up of antigens delivered by pathogens. These M cells at that point alarm the hidden B cells and T cells in the tonsil that a pathogen is available and a resistant reaction is empowered. B cells are initiated and multiply in territories called germinal focuses in the tonsil. These germinal focuses are places where B memory cells are made and secretory immunizer (IgA) is created. They create white platelets to enable your body to battle contamination. The tonsils battle microscopic organisms and infections that enter

your body through your mouth. In any case, tonsils are likewise helpless against contamination from these trespassers. Infections are the most widely recognized reason for tonsillitis.

4. Peyer's patches: Peyer's patches additionally named as totaled lymphoid knobs, or PP are composed lymphoid follicles, named after the Swiss anatomist Johann Conrad Peyer. They are vital piece of gut related lymphoid tissue and are huge masses of intersecting lymphoid follicles, found in the dividers of the ileum, some portion of the small digestive system. Peyer's patches are stretched thickenings of the intestinal epithelium around 100 are found in people. Peyer's patches are described by the follicle-related epithelium, which covers each lymphoid follicles. Follicle-related epithelium varies from run of the mill little intestinal villus epithelium: it has less challis cells along these lines bodily fluid layer is more slender, and it is additionally described by the nearness of particular M cells of Microfold cells, which give take-up and transport of antigens from lumen. The lumen of the gastrointestinal tract is presented to the outer condition; populated with pathogenic microorganisms. Peyer's patches consequently assume an imperative part in the insusceptible control of the intestinal lumen and in encouraging the age of the resistant reaction inside the mucosa. Pathogenic microorganisms and different antigens entering the intestinal tract experience macrophages, dendritic cells, B-lymphocytes, and T-lymphocytes found in Peyer's patches and different destinations of gut-related lymphoid tissue (GALT). Peyer's patches in this manner represent the gastrointestinal framework much as the tonsils represent the respiratory framework, catching outside particles, examining them, and devastating them.

Peyer's patches are secured by an uncommon follicle-related epithelium that contains particular cells called microfold cells (M cells) which gather antigen straightforwardly from the lumen and convey it to antigen-introducing cells. Dendritic cells and macrophages can likewise specifically gather the lumen by expanding dendrites through transcellular M cell-particular pores. In the meantime the paracellular pathway of follicle-related epithelium is shut firmly to counteract entrance of antigens and persistent contact with invulnerable cells. Immune system microorganisms, B-cells and memory cells are animated after experiencing antigen in Peyer's patches. These cells at that point go to the mesenteric lymph hubs where the

insusceptible reaction is intensified. Enacted lymphocytes go into the circulatory system by means of the thoracic channel and go to the gut where they do their last effector capacities. The development of B-lymphocytes happens in the Peyer's fix.

5. Tertiary lymphoid tissue (TLOs): It regularly contains far less lymphocytes, and accept an insusceptible part just when tested with antigens that outcome in aggravation. Tertiary lymphoid organs (TLOs) are collections of lymphoid cells in endless irritation that look like LNs in their cell substance and association, high endothelial venules, and lymphatic vessels (LVs). Albeit intense irritation can bring about inadequate LVs, TLO LVs seem to work ordinarily in that they deplete liquid and transport cells that react to chemokines and sphingosine-1-phosphate (S1P) slopes. It accomplishes this by bringing in the lymphocytes from blood and lymph. TLOs, additionally alluded to as ectopic lymphoid tissues, are gatherings of cells in perpetual irritation and are alluded to as tertiary to recognize them from optional lymphoid organs (SLOs). SLOs emerge amid advancement at enter areas in the body under the control of an exact formative program. TLOs are described by their cell, hierarchical, chemokine, and vascular likeness to SLOs, particularly LNs. These similitudes incorporate T and B cell compartmentalization, APCs, for example, DCs and follicular DCs, stromal cells, courses, and a profoundly composed vascular arrangement of HEVs and LVs.

4.3.3LYMPHATIC SYSTEM

The lymphatic framework is a piece of the circulatory framework and an imperative piece of the invulnerable framework, involving a system of lymphatic vessels that convey unmistakable liquid called lymph towards the heart. The lymphatic framework is a system of tissues and organs that assistance free the group of poisons, squander and other undesirable materials. The essential capacity of the lymphatic framework is to transport lymph, a liquid containing contamination battling white platelets, all through the body. Lymphatic framework, a subsystem of the circulatory framework in the vertebrate body that comprises of an unpredictable system of vessels, tissues, and organs. The lymphatic framework keeps up liquid adjust in the body by gathering abundance liquid and particulate issue from tissues and saving them in the circulatory system. It additionally guards the body against contamination

by providing ailment battling cells called lymphocytes. As blood courses under weight, its liquid segment (plasma) leaks through the thin mass of the vessels into the encompassing tissue. A lot of this liquid, called interstitial liquid, comes back to the blood through the hairlike layers. The rest of the interstitial liquid, now called lymph, streams from the spaces in connective tissue into a system of minor open lymphatic vessels and after that into a progression of continuously bigger gathering vessels called lymphatic vessels. The biggest lymphatic vessel, the thoracic pipe, exhausts into the left subclavian vein close to the heart. Along these lines, the lymphatic framework catches liquid lost from the blood and returns it to the blood, in this way guaranteeing relentless state levels of liquid inside the circulatory framework. The heart does not draw the lymph through the lymphatic framework; rather the stream of lymph is accomplished as the lymph vessels are crushed by developments of the body's muscles. A progression of one-route valves along the lymphatic vessels guarantees that lymph streams just one way. At the point when a remote antigen gets access to the tissues, it is grabbed by the lymphatic framework and is conveyed to different sorted out lymphoid tissues, for example, lymph hubs, which trap the outside antigen. As lymph goes from the tissues to lymphatic vessels, it turns out to be dynamically enhanced in lymphocytes. Along these lines, the lymphatic framework additionally fills in as a methods for transporting lymphocytes and antigen from the connective tissues to sorted out lymphoid tissues where the lymphocytes may collaborate with the caught antigen and experience initiation. The lymphatic framework comprises of lymphatic organs, a leading system of lymphatic vessels, and the circling lymph. Lymphatic tissues start to create before the finish of the fifth seven day stretch of embryonic advancement. Lymphatic vessels create from lymph sacs that emerge from creating veins, which are gotten from mesoderm.

Function: The lymphatic framework assumes a noteworthy part in body's safe framework, as the essential site for cells identifying with versatile resistant framework including T-cells and B-cells. Cells in the lymphatic framework respond to antigens exhibited or found by the cells straightforwardly or by other dendritic cells. At the point when an antigen is perceived, an immunological course starts including the actuation and enrollment of an ever increasing number of cells, the creation of antibodies and cytokines and the enlistment of other immunological cells,

for example, macrophages. The lymphatic framework additionally has numerous interrelated capacities, for example, it is in charge of the expulsion of interstitial liquid from tissues, it ingests and transports unsaturated fats and fats as chyle from the stomach related framework, it transports white platelets to and from the lymph hubs into the bones, the lymph transports antigen-exhibiting cells, for example, dendritic cells, to the lymph hubs where a resistant reaction is empowered.

4.4 SUMMARY

The cells that take part in the safe reaction are white platelets, or leukocytes. The lymphocyte is the main cell to have the immunologic properties of specificity, assorted variety, memory, and self/non-self acknowledgment. Huge numbers of the body's cells, tissues, and organs emerge from the offspring of various immature microorganism populaces. The division of a foundational microorganism can bring about the generation of another undifferentiated organism and a separated cell of a particular sort or gathering. All leukocytes create from a typical multipotent hematopoietic undeveloped cell amid hematopoiesis. Different hematopoietic development factors (cytokines) initiate expansion and separation of the distinctive platelets. The separation of undifferentiated organisms into various cell sorts requires the declaration of various ancestry deciding qualities. Various interpretation factors assume essential parts in such manner. There are three sorts of lymphocytes: B cells, T cells, and common executioner cells (NK cells). NK cells are substantially less plentiful than B and T cells, and most do not have a receptor that is particular for a specific antigen. Be that as it may, a subtype of NK cells, NK1-T cells, have both T-cell receptors and a significant number of the markers normal for NK cells. The three sorts of lymphoid cells are best recognized on the premise of capacity and the nearness of different film atoms. Macrophages and neutrophils are specific for the phagocytosis and corruption of antigens. Basophils and pole cells are non phagocytic cells that discharge an assortment of pharmacologically dynamic substances and assume critical parts in hypersensitive responses. The essential lymphoid organs give destinations where lymphocytes develop and turn out to be antigenically dedicated. T lymphocytes develop inside the thymus, and B lymphocytes emerge and develop inside the bone marrow of people. Essential lymphoid organs are likewise places of

determination where numerous lymphocytes that respond with self antigens are disposed of. Besides, the thymus disposes of thymocytes that would develop into futile T cells in light of the fact that their T-cell receptors can't perceive self-MHC. The lymphatic framework gathers liquid that aggregates in tissue spaces and returns this liquid to the dissemination by means of the left subclavian vein. It additionally conveys antigens to the lymph hubs, which interfere with the course of lymphatic vessels. Auxiliary lymphoid organs catch antigens and give destinations where lymphocytes end up plainly actuated by collaboration with antigens. Actuated lymphocytes experience clonal multiplication and separation into effector cells. There are a few sorts of auxiliary lymphoid tissue: lymph hubs, spleen, the free groups of follicles, and Peyer's patches of the digestive tract, and cutaneous-related lymphoid tissue. Lymph hubs trap antigen from lymph, spleen traps blood-borne antigens, intestinal-related lymphoid tissues (and also other auxiliary lymphoid tissues) cooperate with antigens that enter the body from the gastrointestinal tract, and cutaneous-related lymphoid tissue secures epithelial tissues.

4.5GLOSSARY

Apoptosis: A type of modified cell demise, portrayed by endonuclease assimilation of DNA

B-1/B-2 cells: The two major subpopulations of B lymphocytes.

basophil: A type of granulocyte found in the blood and resembling the tissue mast cell

Bursa of Fabricius: A primary lymphoid organ in avian species, located at the cloacal-hind gut junction; it is the site of B-cell maturation

Chemokines: A family of structurally-related cytokines which selectively induce chemotaxis and activation of leukocytes.

Cortex: Outer (peripheral) layer of an organ.

Cytokines: Low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells

Cytotoxic T lymphocyte (CTL, Tc): T-cells which kill target cells following

recognition of foreign peptide–MHC molecules on the target cell membrane.

Dendritic cell: Refers to an inter-digitating dendritic cell which presents processed antigens to T-cells in the T-cell areas of secondary lymphoid tissues

Effector cells: Cells which carry out an immune function

Gut-associated lymphoid tissue (GALT): Includes Peyer's patches, appendix and solitary lymphoid nodules in the submucosa

Helper T lymphocyte (Th): A subclass of T-cells which provide necessary help for the expression of effector function by other cells in the immune system

Hematopoiesis: The production of erythrocytes, leukocytes and platelets

Hematopoietic stem cells: Self-renewing stem cells that are capable of giving rise to all of the formed elements of the blood

Immunocompetence: The ability of the body to produce a normal immune response following exposure to an antigen.

Interleukins (IL): Designation for some of the cytokines secreted by leukocytes.

Kuppfer cells: Fixed tissue macrophages lining the blood sinuses in the liver.

Leukocyte: White blood cells, which include neutrophils, basophils, eosinophils, lymphocytes and monocytes

Lymph: The tissue fluid which drains into and through the lymphatic system

Macrophage: Large phagocytic cell, derived from the blood monocyte, which also functions as an antigen-presenting cell and can mediate ADCC.

Mast cell: A tissue cell with abundant granules which resembles the blood basophil.

Medulla: Inner (central) region of an organ.

Mucosa-associated lymphoid tissue (MALT): Lymphoid tissue present in the surface mucosa of the respiratory, gastrointestinal and genitourinary tracts.

Peyer's patches: Part of the gut associated lymphoid tissue (GALT) and found as distinct lymphoid nodules mainly in the small intestine

Phagocyte: Cells, including monocytes/macrophages and neutrophils, which are

specialized for the engulfment of cellular and particulate matter

Plasma cell: Terminally differentiated B lymphocyte which actively secretes large amounts of antibody

Primary immune response: The relatively weak immune response which occurs upon the first encounter of naive lymphocytes with a given antigen.

Primary lymphoid organs: The sites at which immune-competent lymphocytes develop, i.e. bone marrow and thymus in mammals

Stem cell: Multi potential cell from which differentiated cells derive

T-cell receptor (TCR): The hetero-dimeric antigen receptor of the T lymphocyte exists in two alternative forms

Thymocyte: Developing T-cell in the thymus.

White pulp: The portion of the spleen containing dense aggregation of lymphocytes, namely lymphoid follicles, and peri-arteriolar white sheath; distinguished from RED-PULP.

4.5 SELF ASSESSMENT QUESTION AND ANSWERS

Multiple Choice Questions

1. B cell are distinguished from T cells by the presence of

   A. CD4 B. Surface Ig

   C. CD8 D. CD3

2. Protection against microorganisms inside cells is provided by:

   A. T-cells B. Antibody

   C. C3b D. C1q

3. Factors may influence the induction of an immune response include

   A. The nature of an antigen B. The route of administration

   C. The dose of antigen D. All of the above

4. The secondary, but not the primary, immune response is based on

   A. Memory B. The bonus effect of multivalency

   C. Complement activation D. Mast cell degranulation

5. T cell-derived cytokines

A. Are antigen-specific products of T cell activation

B. Are stored in the resting T cell and released on activation

C. Are MHC-restricted in their effects

D. Influence the class of antibodies produced by B cell

Answers:

1. Surface Ig
2. T-cells
3. All of the above
4. Memory
5. Influence the class of antibodies produced by B cell

Fill in the blanks:

1. During the_____ thymus reaches its maximal size.

2. _____always act by binding to specific receptors.

3.The lymphatic system consist of_____&_____.

4. T lymphocytes mature in the_____.

5. Bone marrow is of two types i.e. _____bone marrow & _____bone marrow.

Answer:

1. Teenage/puberty
2. Cytokines
3. Lymphatic vessels & lymphoid organs
4. Thymus.
5. Red & yellow

Short Answer Type Questions

ine lymph?

Ans. Lymph is the fluid that circulates throughout the lymphatic system.

2. Name the primary lymphoid organs?

Ans. The thymus and the bone marrow constitute the primary lymphoid organs.

3. Name the secondary lymphoid organs?

Ans. The lymph nodes and the spleen are secondary lymphoid organs.

4. Define tonsils?

Ans.The tonsils are a pair of soft tissue masses located at the rear of the throat (pharynx). Each tonsil is composed of tissue similar to lymph nodes, covered by pink mucosa.

5. Explain MALT?

Ans. MALT stands for mucosa associated lymphoid tissue, the mucosa is a moist tissue that lines body organs and cavities including nose, mouth, lungs, and digestive system.

4.6.5 Long Answers Type Questions

1. Explain the primary lymphoid organs and summarize their functions in the immune response?
2. Explain T lymphocytes & their working?
3. Briefly describe B lymphocytes & its types?
4. Explain the role of lymph nodes in immunity?
5. Describe & explain working of spleen?

4.7 REFERENCES:

Lydyard,P.M., Whelan, A. and Fanger, M.W., (2004). Immunology. Garland Science/BIOS Scientific Publishers Limited

UNIT:5 HUMORALIMMUNITY

CONTENT

5.1OBJECTIVES

- To define antigen & its antigenecity
- To differentiate antigen from immunogens and haptens
- To define antibody & its characteristics
- To explain various types of immunoglobulins
- To describe complement system

5.2ANTIGEN

A foreign substance that is fit for animating an insusceptible reaction, particularly initiating lymphocytes, which are the body's contamination battling white platelets, is named as an antigen. An antigen might be a substance from nature, for example, chemicals, microorganisms, infections, dust or may likewise from inside the body. An antigen is a particle equipped for prompting a safe reaction with respect to the host living being, however once in a while antigens can be a piece of the host itself. An antigen is a protein communicated by a microscopic organisms or infection that is perceived by the safe framework as remote which can empower the creation of antibodies and consolidate particularly with them. Typically an antigen is an atom, maybe on the phone surface of a bacterium or infection. Antigens are constantly 'remote' and trigger an assault. The framework is typically tolerant of its own particles, which don't begin an assault. At the point when an antigen is brought into the body it causes the creation of antibodies. Antigens incorporate microscopic organisms, cells of transplanted organs, plant dust and poisons. Antigens fortify the creation of antibodies: they don't deliver them specifically. Whenever a similar antigen contacts the body, a full-scale insusceptible reaction isn't required as the body as of now has a particular immunizer accessible in a flash for that antigen. Substances, for example, proteins, nucleoproteins, polysaccharides and glycolipids can go about as antigens. Proteins are known to be the most powerful antigens and polysaccharides are second in the rundown. The first occasion when that another antigen comes into contact with the body the reaction of the resistant framework will be an entire invulnerable reaction. Amid this first reaction, the antigen will make antibodies be made. Lipids and nucleic acids are antigenic just when joined with proteins and polysaccharides. On the surface of antigens are locales, called antigenic determinants that fit and tie to receptor atoms of

corresponding structure on the surface of the lymphocytes. The official of the lymphocytes' receptors to the antigens' surface atoms fortifies the lymphocytes to increase and to start an invulnerable reaction—including the generation of neutralizer, the actuation of cytotoxic cells, or both—against the antigen. The measure of counter acting agent framed in light of incitement relies upon the kind and measure of antigen included the course of section to the body, and individual qualities of the host.

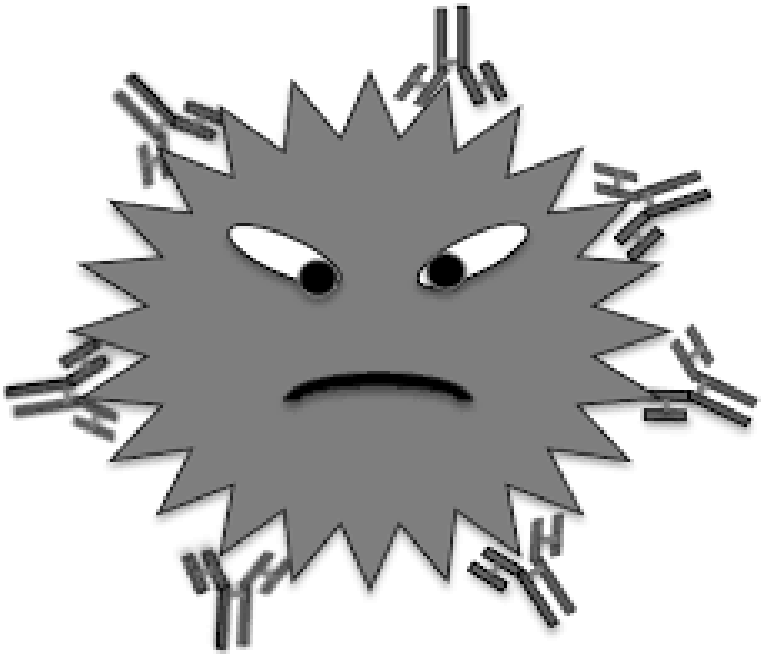


Fig 5.1Antigens can be classified according to their source and are of following types:

5.2.1EXOGENOUS ANTIGENS

Exogenous antigens will be antigens that have entered the body all things considered, by inward breath, ingestion or infusion. The invulnerable framework's reaction to exogenous antigens is frequently subclinical. By endocytosis or phagocytosis, exogenous antigens are taken into the antigen-displaying cells (APCs) and prepared into sections. APCs at that point show the pieces to T aide cells (CD4+) by the utilization of class II histocompatibility atoms on their surface. Some T cells are particular for the peptide: MHC complex. They end up plainly initiated and begin to discharge cytokines, substances that actuate cytotoxic T lymphocytes (CTL), immunizer emitting B cells, macrophages and different particles. A few antigens begin as exogenous, and later end up noticeably endogenous for instance, intracellular infections. Intracellular antigens can be come back to course upon the pulverization of the contaminated cell.

5.2.3ENDOGENOUS ANTIGENS

Endogenous antigens are created inside ordinary cells because of typical cell digestion, or as a result of viral or intracellular bacterial contamination. The parts are then introduced on the cell surface in the complex with MHC class I particles. In the event that enacted cytotoxic CD8+ T cells remember them, the T cells discharge different poisons that reason the lysis or apoptosis of the tainted cell. Endogenous antigens incorporate xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens.

5.2.4NEOANTIGENS

Neoantigens are those that are completely truant from the typical human genome. As contrasted and nonmutated self-antigens, neoantigens are of pertinence to tumor control, as the nature of the T cell pool that is accessible for these antigens isn't influenced by focal T cell resistance.

5.2.5TUMOUR ANTIGENS

Tumor antigens are those antigens that are displayed by MHC class I or MHC class II atoms on the surface of tumor cells. Antigens discovered just on such cells are called tumor-particular antigens (TSAs) and by and large outcome from a tumor-particular transformation. More typical are antigens that are introduced by tumor cells and ordinary cells, called tumor-related antigens (TAAs). Cytotoxic T lymphocytes that perceive these antigens might have the capacity to devastate tumor cells. Tumor antigens can show up on the surface of the tumor as, for instance, a changed receptor, in which case they are perceived by B cells.

5.2.6 VIRAL ANTIGENS

Virus-related tumors, for example, cervical growth and a subset of head and neck diseases, epitopes got from viral open perusing outlines add to the pool of neoantigens.

In light of hereditary thought antigens are partitioned into three sorts: Autoantigens, alloantigens and heteroantigens

- **Autoantigens:** These are simply the antigens having a place with have, any antigen that fortifies autoantibodies in the living being that delivered it.

- **Alloantigens:** These are the antigens got from different individuals from types of the host, however not from the host itself. Such antigens are vital in tissue transplant and blood transfusion forms.

  e.g antigens display on giver and the beneficiary RBCs are alloantigens to each other
- **Heteroantigens:** These antigens are from two unique species, for example, plants and creatures or microorganisms and so forth. A counter acting agent that is created by a person of one animal category and is equipped for empowering a resistant reaction in a person of another species.

5.2.7ANTIGENICITY

Antigen is the limit of a concoction structure to tie particularly with a gathering of specific items that have versatile resistance: T cell receptors or antibodies. Antigenicity is the capacity to join particularly with the antiboby and every single surface receptor. Antigens are the substances which respond with the results of an invulnerable reaction, the effector atoms (Ab)/effector cells (CTLs) for end of remote particles and this property is known as antigenicity. Consequently an antigen may tie particularly perfectly or B cell receptor, yet not prompt a versatile insusceptible reaction. On the off chance that the antigen induces a reaction, it is an 'immunogenic antigen', which is alluded to as an immunogen. In spite of the fact that a substance that incites a particular resistant reaction is generally called an antigen, it is all the more suitably called an immunogen. Substances fit for prompting a particular insusceptible reaction are called antigens. To be more exact the substances which can produce an insusceptible reaction (humoral as well as cell interceded) are called immunogens and this capacity of a substance is called immunogenicity. Albeit all particles that have the property of immunogenicity additionally have the property of antigenicity, yet the invert isn't genuine i.e. all immunogens are antigen yet all antigensare not immunogen. Immunogenicity and antigenicity are connected however particular immunologic properties. The littlest unit of antigenicity is known as the antigenic determinant or epitope.

- **Detrminants of antigenicity**

  Immunogenicity/Antigenicity isn't an inborn property of an antigen yet rather relies upon various properties of the specific natural framework that the antigen experiences. Various properties are in charge of making a substance antigenic which are as per the following:

- **Foreignness:** Most critical factor of an immunogen is that it must be outside as for its host. To inspire a resistant reaction, a particle must be perceived as nonself by the natural framework. Just antigens which are 'remote' to the individual (nonself) initiate an invulnerable reaction. The antigenicity of a substance is identified with the level of its strangeness. Self antigen are not perceived because of people are tolerant to their own self atom. A lot of their capacity to endure self antigens emerges amid lymphocyte improvement, amid which youthful lymphocytes are presented to self-segment. At the point when the antigen is brought into a living being the level of immunogenicity relies upon the level of its strangeness. Antigens from related species are less antigenic than those from removed species.

  Example: Antigen Bovine Serum Albumin (BSA) isn't immunogenic when infused into a dairy animals yet it is firmly immunogenic when infused into rabbit.
- **Molecular measure:** Antigenicity is identified with the sub-atomic size, as there is a connection between the span of immunogen and immunogenicity. Most antigens are expansive, complex atoms with a sub-atomic weight by and large more noteworthy than around 100,000 daltons. Substances with under 5000 - 10,000 dalton measure are poor immunogens. When all is said in done expansive atoms are better immunogens when contrasted with littler particles. Large atoms are exceptionally antigenic and particles with low antigenicity are nonantigenic.

  Example: Bovine gamma globulin (BCG) 1,50,00 dalton, Bovine serum egg whites (BSA) 69,0000 dalton, Tetanus toxoid 1,50,000 dalton, Ovalbumin (OVA) 44, 000 dalton.
- **Chemical piece:** The more prominent the level of unpredictability of protein the most enthusiastic will be safe reaction in these manner proteins and polysaccharides are great immunogen when contrasted with lipids and nucleic acids as most basic resistant reaction are those to proteins. Copolymers made out of various amino acids or sugars are normally more immunogenic than homopolymers of their constituents. Exceedingly adaptable particles having no settled shape are poor antigens. Straightforward dull polysaccharides don't accept a steady arrangement thus is poor immunogens.

  Example: gelatin, flagellin
- **Degardability:** The T partner cells (Th) cells are required to be enacted and help in the advancement of both the humoral and cell intervened resistant reaction. Since an

antigen is to be prepared and given MHC particles for improvement of invulnerable reaction, the macromolecules that can't be debased and displayed are poor immunogens. Which can’t be debased and handled by the degradative chemicals of the antigen displaying cells (APCs) fill in as poor immunogens? Vast particles are promptly phagocytosed and handled and henceforth are more immunogenic.

e.g., Polymers containing D-amino acids

5.3 ANTIGEN PROCESSING AND PRESENTATION

Just substances which are processed and are powerless to the activity of tissue proteins act as antigens. Antigens brought into the body are corrupted by the host into pieces of fitting size containing the antigenic determinants. The advancement of both humoral and cell-intervened invulnerable reactions requires collaboration of T cells with antigen that has been prepared and given together MHC particles. Expansive, insoluble macromolecules are unequivocally more immunogenic than little, dissolvable atoms on the grounds that the bigger particles are all the more eaisly phagocytosed and prepared by APC. Macromolecules that can't be corrupted and given MHC particles are poor immunogens.

Example: As polymers of D amino corrosive can't be corrupted on the grounds that APC can debase just protein containing L-amino corrosive.

- **Dosage and course of organization:** A deficient dosage and an extreme high measurement go about as poor antigens. Lower measurements can either neglect to actuate enough lymphocytes or they incite a nonresponsive state. Too high a measurement likewise neglects to incite an insusceptible reaction since it instigates a nonresponsive state in the lymphocytes. Along these lines a negligible ideal measurement and furthermore a rehashed organization (supporter dosages) are required to animate a solid safe reaction by expanding the multiplication of particular T and B cells. The course of organization impacts the cells and organs engaged with an insusceptible reaction. Normal courses of organization are intravenous, intradermal, subcutaneous, intramuscular and intraperitoneal. It is the course of organization which chooses the safe organs and cell populaces which will partake in the safe reaction.
- **Genotype of the Recipient:** The hereditary constitution (genotype) of an inoculated creature impacts the kind of safe reaction the creature shows, and additionally the

level of the reaction. The qualities that encode MHC, B cell and T cell receptors and different proteins for invulnerable direction are known to impact the sort and the force of the resistant reaction.

- **Antigenicity specificity:** The premise of antigenic specificity is a stereochemical .Crossreaction can happen between antigens that bear stereochemical likenesses. In a few occasions, clear cross responses may really be because of the sharing of indistinguishable antigenic determinants by various antigens.
- **Species specificity:** Tissues of all people in animal varieties contain animal types particular antigens. There exits some level of cross - response between antigens of related species.
- **Isospecificity:** Isoantigens are antigens found in a few however not all individuals from an animal categories. The species might be gathered relying upon the nearness of various iso-antigens in its individuals.
- **Autospecificity:** Autologous or self antigens are conventionally non-antigenic however there are exemptions. Sequestrated antigens that are not regularly discovered free available for use or tissue liquids are not perceived as a self antigens.. Thus, antigens that are truant amid embryonic life and grow later are additionally not perceived as self antigens.
- **Organ specificity:** Some organs, for example, the mind, kidney and focal point protein of various species, share a similar antigen. Such antigens, normal for organ or tissue and found in various species, are organ – particular antigens.
- **Heterogenetic (heterophile) specificity:** The same or firmly related antigens may sometimes happen in various organic species, classes and kingdoms. These are known as heterophile antigens.

5.5ADJUVANTS

Adjuvants (Latin adjuvare, to upgrade) are substances that, when blended with an antigen and infused with it, improve the immunogenicity of that antigen. An adjuvant is a pharmacological or immunological operator that adjusts the impact of different specialists. Adjuvants are frequently used to help the insusceptible reaction when an antigen has low immunogenicity or when just little measures of an antigen are accessible. Adjuvants might be added to an immunization to alter the insusceptible reaction by boosting it, for example, to give a higher measure of antibodies and a more drawn out enduring insurance, in this way

limiting the measure of infused outside material. Adjuvants are utilized to improve the viability of an immunization by altering the insusceptible reaction to specific sorts of invulnerable framework cells, by actuating T cells rather than immunizer discharging B cells relying upon the motivation behind the antibody. Adjuvants are likewise utilized as a part of the creation of antibodies from vaccinated creatures. They apply following impacts; antigen industriousness is delayed. Co-stimulatory signals are improved. Neighborhood irritation is expanded. The nonspecific expansion of lymphocytes is fortified. Albeit immunological adjuvants have generally been seen as substances that guide the safe reaction to the antigen, adjuvants have additionally advanced as substances that can help in settling details of antigens, particularly for antibodies managed for creature wellbeing. Adjuvants are added to immunizations to fortify the safe framework's reaction to the objective antigen, however don't give insusceptibility themselves. Adjuvants can act in different routes in exhibiting an antigen to the resistant framework. Adjuvants can go about as a station for the antigen, introducing the antigen over a more extended timeframe, along these lines expanding the insusceptible reaction before the body clears the antigen. An adjuvant can likewise go about as an aggravation, which draws in and intensifies the body's invulnerable reaction.

Example: Alum is the most usually utilized adjuvant in human inoculation. It is found in various antibodies, including diphtheria-lockjaw pertussis, human papillomavirus, and hepatitis immunizations.

Adjuvants are ordered into two sorts as following:

1. **Particulate adjuvants**: Those adjuvants which exist as tiny particles and owe at least some of their adjuvant action. These contain-

    - Aluminium salts
    - Oil-in-water emulsions
    - Water-in-oil emulsions
    - Nano and microparticles
    - Liposomes
    - Immune stimulating complexes (ISCOM adjuvant-Iscoter AB)

2. **Non-Particulate adjuvants:** These are adjuvants where action does not rely on any particulate or multimeric nature. They are general immunomodulators through some enhanced focusing on, most advantage from relationship with a particulate adjuvant. These incorporate:

- Muramyl dipeptide (MDP) and erivatives
- Non-ionic block copolymers
- Saponin
- Bacterial toxins
- Derivatized polysaccharides
- Carbohydrate polymers
- Cytokines
- Lipid A

5.5.1MODES OF ACTION OF ADJUVANTS

Adjuvants are expected to enhance steering and versatile insusceptible reactions to antigens. These responses are interceded by two fundamental sorts of lymphocytes, B and T cells. Adjuvants apply their belongings through five distinctive specified instruments.

5.5.2IMMUNOMODULATION

It alludes to the capacity of numerous adjuvants to change the cytokine organize i.e. just immunomodulatory mixes will apply an adjuvant impact when presented at a different time or site to the immunogen. Immunomodulation may bring about a general up-direction of the whole safe framework.Determination of a proper immunomodulatory adjuvant prompt an upgraded safe reaction. A decent adjuvant improves a balanced invulnerable reaction.

Presentation: This alludes to the capacity of an adjuvant to safeguard the conformational respectability of an antigen and to show this to suitable resistant effector cells. This will happen when an adjuvant can collaborate with an antigen such that conformational epitopes are all the more effec¬tively kept up.

5.5.3INDUCTION OF CD8 +CYTOTOXIC T-LYMPHOCYTE (CTL) RESPONSES

This alludes to enlistment of CTL reactions by and large expects antigen to be prepared inside the cell cytosol where peptides, for the most part of nine amino acids 9 mers, wind up plainly joined inside the shut end score of the MHC class I atom and are then communicated on the cell surface.

5.5.4TARGETING

This characterizes the capacity of an adjuvant to convey an im¬munogen to invulnerable effector cells, for the most part by means of APCs. This type of adjuvant action may not change the kind of invulnerable reaction yet rather will influence the measure of immunogen required to accomplish a given impact i.e. the proficiency of the age of the resistant reaction.

5.5.5DEPOT GENERATION

It is accomplished as a short and long haul station, by giving a ceaseless or beat discharge. Here and now terminals are accomplished by aluminum salts and water-oil emul¬sions, where antigen is caught at the infusion site and accordingly can't be lost by liver freedom. Extraction of the infusion site eight to ten days subsequent to dosing has pretty much nothing if any impact on extent or span of reaction, suggest¬ing that antigen has either been evacuated or walled-off by that stage. Long haul stations are best accomplished utilizing engineered polymers, for example, polylactide coglycolide (PLG), to create microspheres, which corrupt to yield a beat conveyance.

5.6HAPTENS

Haptens are little natural particles that are antigenic however not immunogenic. These are little particles that can tie to antibodies yet can't without anyone else initiate an insusceptible reaction. They can inspire a safe reaction just when appended to a vast transporter, for example, a protein. These mixes end up plainly immunogenic when they are conjugated with atomic physio-substance complex transporter and change over into immunogenic hapten bearer conjugate. Once the body has created antibodies to a hapten-transporter, the conjugate shaped by coupling a hapten to a substantial bearer protein is immunogenic and evokes generation of hostile to hapten antibodies when infused into a creature. Such infusions likewise deliver hostile to bearer and against hapten/transporter antibodies too. Antibodies created against haptens are particular for transporters, the haptens and consolidated parts of both haptens and bearer. Independent from anyone else a hapten can't work as an

immunogenic epitope yet when numerous particles of a solitary haptens are coupled to a transporter protein, the hapten winds up plainly accessable to the insusceptible framework and capacity as immunogen.

Example: Home-pregnancy test unit contain antihapten antiboby to decide if a lady's pee contain HCG (Human endless gonadotropin)

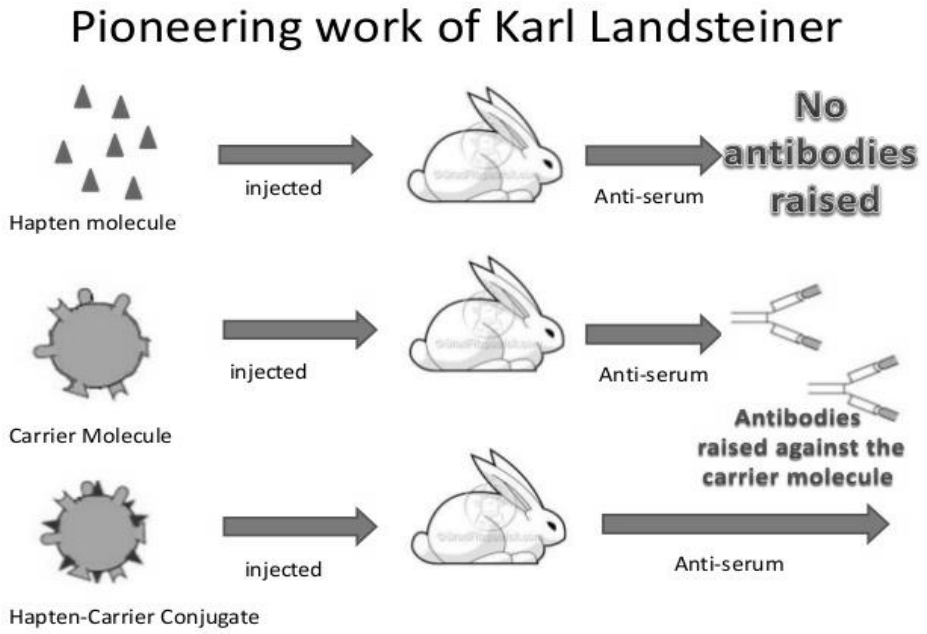
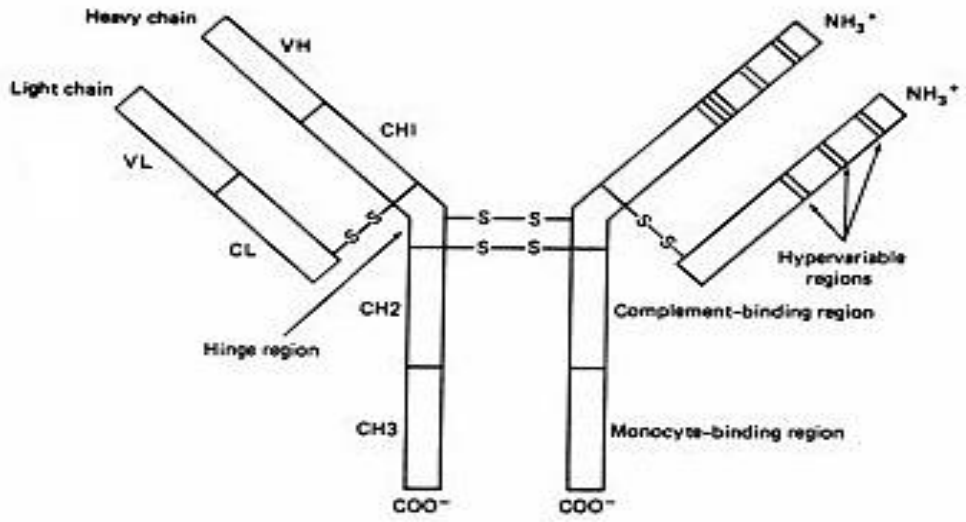


Fig 5.2 Pioneering work of karl Landsteiner

5.7IMMUNOGLOBULIN'S

The immunoglobulin's get their name from the finding that they move with globular proteins when counter acting agent containing serum is put in an electrical field. An immunoglobulin (Ig), otherwise called a counter acting agent (Ab), is a vast, Y-molded protein created chiefly by plasma cells that is utilized by the safe framework to distinguish and kill pathogens. Antibodies are antigen restricting proteins show on the B-cell film and discharged by plasma cells. The counter acting agent atom is discharged into the blood or lymph because of an antigenic boost, for example, a bacterium, infection, parasite, or transplanted organ, and that kills the antigen by restricting particularly to it. They are the glycoprotein with particular amino corrosive grouping and distinctive antigen restricting destinations. Antibodies by and large are called as Immunoglobulins i.e. Ig. It is the most inexhaustible protein segment in blood. Immunizer atom performs two particular capacities antigen acknowledgment and antigen end. Antibodies are emitted by B cells of the versatile safe framework, for the most

part by separated B cells called plasma cells. Antibodies can happen in two physical structures, a solvent frame that is discharged from the cell to be free in the blood plasma, and a layer bound shape that is connected to the surface of a B cell and is alluded to as the B-cell receptor (BCR).



5.5.8STRUCTURE

Blood can be isolated in an axis into a liquid and a cell division. The liquid portion is the plasma and the cell part contains red platelets, leukocytes, and platelets. Plasma contains the greater part of the solvent little atoms and macromolecules of blood, including fibrin and different proteins required for the development of blood clumps. In the event that the blood or plasma is permitted to cluster, the liquid stage that remaining parts is called serum, antibodies lives in the serum. The least complex antibodies are Y-formed atoms with two indistinguishable antigen restricting site, one at the tip of each arm. Antibodies have a typical structure of four polypeptide chains. This structure comprises of two indistinguishable light (L) chains, polypeptides of around 25,000 sub-atomic weight, and two indistinguishable substantial (H) chains, bigger polypeptides of sub-atomic weight at least 50,000. Like the counter acting agent atoms they constitute, H and L chains are additionally called immunoglobulins. Each light bind is bound to a substantial chain by a disulfide bond, and by

such non-covalent connections as salt linkages, hydrogen bonds, and hydrophobic bonds, to shape a heterodimer (H-L). Comparable non-covalent communications and disulfide spans interface the two indistinguishable overwhelming and light (H-L) fasten blends to each other to frame the essential four-chain (H-L)2 neutralizer structure, a dimer of dimmers. Every polypeptide chain contains an amino terminal and a carboxyl terminal. Antibodies are glycoproteins having a place with the immunoglobulin superfamily. They constitute the vast majority of the gamma globulin portion of the blood proteins. They are regularly made of fundamental basic units—each with two huge substantial chains and two little light chains. There are a few distinct sorts of counter acting agent overwhelming chains that characterize the five unique sorts of crystallisable pieces (Fc) that might be connected to the antigen-restricting sections. The five distinct sorts of Fc areas enable antibodies to be assembled into five isotypes. Each Fc locale of a specific counter acting agent isotype can tie to its particular Fc Receptor (aside from IgD, which is basically the BCR), accordingly permitting the antigen-neutralizer complex to intervene distinctive parts relying upon which FcR it ties. The capacity of an immunizer to tie to its relating FcR is additionally regulated by the structure of the glycan(s) introduce at saved destinations inside its Fc region.The capacity of antibodies to tie to FcRs coordinates the proper resistant reaction for each unique kind of outside question they experience. In spite of the fact that the general structure of all antibodies is fundamentally the same as, a little area at the tip of the protein is to a great degree variable, permitting a large number of antibodies with somewhat unique tip structures, or antigen-restricting destinations, to exist. This district is known as the hypervariable locale. Each of these variations can tie to an alternate antigen. This gigantic decent variety of counter acting agent paratopes on the antigen-restricting parts enables the resistant framework to perceive a similarly wide assortment of antigens.

Light chain sequencing: The amino terminal segment of the atom is a piece of the variable or V area and carboxyl terminal is the steady or C locale. Amino terminal locale has 110 a.a. that fluctuates extraordinarily among antibodies of various specificity. The carboxyl terminal portion of the atom called as the steady area has premise of which light chains are of two sort kappa (ќ) and lambda (λ). Anybody individual of an animal categories deliver the two sorts of light chain. In people 60% of light chains are kappa and 40% are lambda.

Heavy chain sequencing: The amino terminal piece of the substantial chain comprises of 100-110 a.a. The rest of the piece of the overwhelming chain or consistent piece of substantial chain indicates five fundamental grouping design, as per which overwhelming

chains are of five distinct sorts and each of these five diverse overwhelming chains are called as an isotype. The length of the steady district is approx. 330 a.a. to 440 a.a. The alpha, beta and gamma contain an expanded peptide succession between steady overwhelming locale 1 and consistent substantial area 2 called as pivot district. Pivot district fill in as a kind of string that permits the Fab segments and Fc locale to move with respect to each other. Pivot locale is rich in proline substance i.e. why it is presented more to catalysts and chemicals. The quaternary structure of the immunoglobulins is framed by encouraging non-covalent communications between immunoglobulin spaces over the characteristics of the β sheets. These collaborations frame interface between non-indistinguishable spaces and between indistinguishable areas.

5.7.2Function

Antibodies perceive the antigen and take an interest in an expansive scope of other natural exercises, which will bring about expulsion of the antigen and demise of the pathogen. The variable districts of the counter acting agent tie to the antigen and the substantial chain steady area includes in an assortment of community communications with different proteins, cells and tissues that outcome in the effector elements of the humoral reaction. These effector capacities result from collaborations between overwhelming chain consistent locales and other serum proteins or cell film receptors.

The principle capacity of counter acting agent activity incorporates the accompanying:

- Neutralisation, in which killing antibodies piece parts of the surface of a bacterial cell or virion to render its assault incapable
- Agglutination, in which antibodies "stick together" outside cells into clusters that are alluring focuses for phagocytosis
- Precipitation, in which antibodies "stick together" serum-solvent antigens, compelling them to accelerate out of arrangement in bunches that are appealing focuses for phagocytosis
- Complement actuation (obsession), in which antibodies that are hooked onto an outside cell urge supplement to assault it with a film assault complex, which prompts the lysis of the remote cell and support of irritation by chemo tactically drawing in provocative cells.

5.7.3Types

In people there are five artificially and physically unmistakable classes of antibodies (IgG, IgA, IgM, IgD, IgE). Distinctive immune response classes with various organic exercises have developed to manage antigens (e.g. organisms) with various properties and which enter the body at various destinations – through the skin, the gastrointestinal or the genitourinary tracts.Each class is recognized by one of a kind amino corrosive arrangements in the substantial chain consistent district that give class-particular basic and utilitarian properties.

(i) Immunoglobulin G (IgG): It is the most bottomless counter acting agent in serum and constitutes around 75-80% of the aggregate serum immunoglobulin. It has the longest half existence of 23-25 days. The IgG particle comprises of two γ substantial chains and two κ or two λ light chains. There are four human IgG subclasses, recognized by contrasts in affix succession and numbered to their diminishing normal serum fixations: IgG1, IgG2, IgG3, and IgG4. The amino corrosive groupings that recognize the four IgG subclasses are encoded by various germ-line CH qualities, whose DNA successions are 90%– 95% homologous. The auxiliary qualities that recognize these subclasses from each other are the span of the pivot locale and the number and position of the between chain disulfide bonds between the overwhelming chains. The unpretentious amino corrosive contrasts between subclasses of IgG influence the organic action of the atom:

- IgG1, IgG3 and IgG4 give invulnerability to the creating baby since they can promptly cross the placenta and go into neonatal dissemination.
- IgG1 and IgG3 tie with high fondness to Fc receptors on the phagocytic cells and in this manner intervene phagocytosis. IgG4 has direct fondness for Fc receptors while IgG2 has a to a great degree low liking.
- IgG3 is the best supplement activator, trailed by IgG1; IgG2 is less effective and IgG4 can't enact supplement by any means.
- IgG assumes a noteworthy part in balance of poisons since it can without much of a stretch diffuse into extravascular spaces.

(ii) **Immunoglobulin M (IgM):** IgM is the main immunoglobulin class delivered in an essential reaction to an antigen, and it is additionally the principal immunoglobulin to be combined by the neonate. IgM represents 5-10% of the aggregate serum immunoglobulin. IgM is discharged by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds. The five monomer subunits are organized with their Fc areas in the focal point of the pentamer and the ten antigen restricting destinations on the fringe of the atom. Each pentamer contains an extra polypeptide called the "J" (joining) chain, which is connected by two of the ten carboxyl terminal spaces. The J chain is required for the polymerization of the monomers to shape pentameric IgM. Monomeric IgM is additionally communicated as film bound neutralizer on B cells. Pentameric IgM has 10 antigen-restricting locales consequently it is more proficient than different isotypes in restricting antigens with many rehashing epitopes, for example, viral particles. Less IgM than IgG is required to kill viral infectivity. IgM is likewise more productive than IgG at enacting supplement. Supplement initiation requires two Fc districts in closeness, and the pentameric structure of single IgM atoms satisfies this necessity. IgM particle does not diffuse well due to the vast size, in this way is found in low focuses in intercellular tissue liquids. J chain encourages the official of IgM to receptors on secretary cells, which transports it crosswise over epithelial linings to enter the outer emissions that submerge mucosal surfaces.

(iii) **Immunoglobulin A (IgA):** It is the overwhelming immunoglobulin class in outside emission, for example, spit, tears, bosom drain, respiratory discharge, and genitourinary and stomach related track discharges. IgA constitutes 10-15% of the aggregate immunoglobulin in serum. IgA exists essentially as a monomer, however some of the time polymeric structures (dimers, trimers and some tetramers) containing J chain polypeptide is additionally observed. The secretory IgA comprises of a dimer or tetramer, a J chain polypeptide and a secretory segment. The J chain polypeptide in IgA encourages polymerization work like if there should arise an occurrence of IgM. The secretory part is created by epithelial cells of mucous layers. The secretory part is comprised of five immunoglobulin like spaces that ties to the Fc area of IgA dimer. The everyday creation of secretory IgA is more noteworthy than that of some other immunoglobulin class. IgA discharging plasma cells are focused along mucous layer surfaces. The plasma cells that create IgA specially move to subepithelial tissue,

where secretory IgA ties to poly-Ig receptor introduce on the basolateral surface of most mucosal epithelia. After IgA ties to poly-Ig receptor, the receptor-IgA complex is then transported over the epithelial cell to luminal film by receptor intervened endocytosis. The poly-Ig receptor is then divided enzymatically from the film and turns into the secretory part, which is bound to and discharged together with polymeric IgA into mucosal emission. The secretory segment veils the destinations helpless to protease cleavage along these lines enabling the polymeric IgA to exist longer in the protease rich mucosal condition. Pentameric IgM is additionally transported into mucosal emission by this instrument. Polymeric secretory IgA helps in catching the pathogen like microscopic organisms and infections by keeping their connection to the epithelial cell divider. Secretory IgA can cross-interface extensive antigens with different epitopes in this way repressing viral disease and bacterial colonization. Buildings of secretory IgA and antigen are effectively ensnared in mucous and afterward killed by ciliated. Epithelial cells of the respiratory track or by peristalses of the gut. Secretory IgA gives powerful barrier against microorganisms like Salmonella, Vibrio cholerae, and Neisseria gonorrhoeae and infections, for example, flu, polio and reovirus (infections have dsRNA genomes). Secretory IgA in bosom drain secures the infant against disease amid the main month of life on the grounds that the insusceptible arrangement of newborn children isn't completely practical.

(iv) Immunoglobulin E (IgE): The serum grouping of IgE is low (0.3µg/ml) however it is exceptionally strong in real life. It intervenes the unfavorably susceptible response or excessive touchiness responses like asthma, roughage fever and hypersensitivity responses. IgE ties to the Fc receptors on the films of basophils and pole cells. Cross linkage of receptor bound IgE by antigen (allergen) instigates basophils and pole cells to translocate their granules to the plasma layer and discharge their substance to the additional cell condition, process known as degranulation. Hence an assortment of pharmacologically dynamic go betweens like histamine, bradykinin and other vasoactive go betweens are discharged and offer ascent to unfavorably susceptible and hypersensitivity signs.

(v) Immunoglobulin D (IgD): It is found on B-cell surface alongside IgM and go about as a receptor for antigen authoritative. It is available in low fixation (30µg/ml) and

constitutes around 0.2% of the aggregate immunoglobulin in serum. It initiates the B cells subsequent to authoritative to the antigen. No other capacity is known about IgD.

5.7COMPLEMENT SYSTEM (ANTIGEN-ANTIBODY REACTIONS)

The expression "supplement" was instituted by Paul Ehrlich to depict the movement in serum, which could "supplement" the capacity of particular neutralizer to cause lysis of microbes. Supplement alludes to new serum fit for lysing neutralizer covered cells. The supplement framework is a piece of the resistant framework that improves (supplements) the capacity of antibodies and phagocytic cells to clear microorganisms and harmed cells from a life form, advances aggravation, and assaults the pathogen's plasma layer. It is a piece of the natural safe framework, which isn't versatile and does not change through the span of a person's lifetime. It can be enlisted and brought without hesitation by the versatile safe framework. Supplement framework is made out of more than 25 unique proteins delivered by hepatocytes, macrophages and intestinal epithelial cells. Fibroblasts and intestinal epithelial cells make C1, while the liver makes C3, C6, and C9. They are available in the dissemination as idle atoms. The supplement framework comprises of various little proteins found in the blood, when all is said in done orchestrated by the liver, and typically coursing as dormant antecedents (genius proteins). At the point when empowered by one of a few triggers, proteases in the framework sever particular proteins to discharge cytokines and start an opening up course of further cleavages. The final product of this supplement initiation or supplement obsession course is incitement of phagocytes to clear outside and harmed material, intermediary irritation to draw in extra phagocytes, and enactment of the cell-murdering layer assault complex. More than 30 proteins and protein sections make up the supplement framework, including serum proteins, serosal proteins, and cell film receptors. They represent around 10% of the globulin portion of blood serum and can fill in as opsonins. Initiation of supplement brings about the generation of a few naturally dynamic atoms, which add to nonspecific invulnerability and irritation. Supplement isn't antigen-particular and it is enacted quickly within the sight of pathogen, so it is considered piece of intrinsic insusceptibility. Since neutralizer likewise initiates some supplement proteins, supplement actuation is additionally part of humoral resistance. Their actuation continues by means of various pathways in a course mold prompting lysis.

5.8.1PATHWAYS OF COMPLEMENT SYSTEM

The supplement actuation can be separated into three pathways, traditional, lectin (mannose restricting protein) and option.

- **Classical Pathway/Antigen-Antibody responses:** Complement actuation by the established pathway usually starts with the arrangement of solvent antigen-counter acting agent com-plexes (invulnerable buildings) or with the authoritative of neutralizer to antigen on an appropriate target, for example, a bacterial cell. IgM and certain subclasses of IgG (human IgG1, IgG2, and IgG3) can enact the traditional supplement pathway. The underlying phase of actuation includes C1, C2, C3, and C4, which are available in plasma in practically latent structures. The development of an antigen-counter acting agent complex instigates conformational changes in the Fc segment of the IgM mole-cule that uncover a coupling site for the C1 part of the supplement framework. C1 is the primary supplement segment to take an interest in traditional pathway. It is made out of C1q, C1r and C1s. Official of C1q to Ag-Ab edifices brings about autocatalysis of C1r. The adjusted C1r severs C1s and this cleavedC1s is fit for dividing both C4 and C2. Actuated C1s enzymatically separates C4 into C4a and C4b. C4b ties to the Ag-bearing molecule or cell film while C4a remains a naturally dynamic peptide at the response site. C4b ties C2, which ends up plainly helpless to C1s and is cut into C2a and C2b. C2a remains complexed with C4b though C2b is discharged. C4b2a complex is known as C3 convertase. C3 convertase, within the sight of Mg++, severs C3 into C3a and C3b. C3b ties to the layer to shape C4b2a3b complex though C3a stays in the microenvironment. A C4b2a3b complex capacity as C5 convertase, which separates C5 into C5a and C5b. Age of C5 convertase marks the finish of the traditional pathway. C5b starts the arrangement of film assault complex. C1qrs can likewise tie to various specialists including some retroviruses, mycoplasma, poly-inosinic corrosive and collected IgG, and start the traditional pathway.

5.9SUMMARY

All immunogens are antigens however not all antigens are immunogens. A substance that actuates a resistant reaction is called an antigen. In the event that the antigen invigorates generation of an immunizer, it will respond particularly, for the most part in a detectable way, with counter acting agent. An immunogen is a substance that can prompts a resistant reaction however which does not really tie to its particular counter acting agent. Immunogenicity is

dictated by many variables including strangeness, atomic size, concoction organization, many-sided quality, dosage, powerlessness to antigen preparing and introduction, the genotype of the beneficiary creature (specifically, its MHC qualities), course of organization, and adjuvants. Most antigens are unfamiliar to the host. They are expansive atoms, for example, proteins and polysaccharides. Little compound gatherings on the antigens particles, called epitopes, constitute that are perceived by antibodies. Haptens are little atoms that can tie to antibodies however a resistant reaction can't without anyone else's input initiate. In any case, the conjugate framed by coupling a hapten to an expansive transporter protein is immunogenic and inspires generation of hostile to hapten antibodies when infused into a creature. Such infusions likewise create hostile to transporter and against hapten/bearer antibodies too. The term immune response means the presence of discrete body that can demonstration against pathogen or its item. Different names incorporate g-globulin, immunoglobulin. Antibodies are safeguard protein (glycoproteins) delivered by versatile insusceptible arrangement of vertebrate to battle attacking pathogens. Neutralizer is a Y molded particle made up of four polypeptides i.e. two chains of higher sub-atomic weight (substantial chain) and two chains of low sub-atomic weight (light chain). N-terminal area of around 110 amino acids in both light and overwhelming chain contrast (fluctuate) among various antibodies and is called as factor district. This locale frames arms of Y molded immunizer and ties antigen. The tail of Y is constituted by consistent area and is engaged with receptor authoritative and supplement initiation. Antibodies have been isolated into various classes or isotypes in view of the distinction in the steady district. The classes IgG, IgA, IgM, IgD, IgE have diverse utilitarian properties. Immunoglobulins of same class showing allelic contrast between themselves, even of maybe a couple amino acids are called as allotypes. Allotypes of IgG in person A will be unique in relation to IgG in person B. Idiotype determinants are framed generally by amino corrosive succession of hypervariable locale of antigen restricting site of counter acting agent. IgG is the most plenteous immunizer of inward body liquids. It battles organisms and their poisons. IgA is overwhelming counter acting agent in outside emissions where it shields surface against viral and bacterial attack. IgM is extremely powerful agglutinator, while IgE is significant immune response of hypersensitive responses. IgD is fundamentally found on the surface of develop B-cell and is most likely associated with lymphocyte initiation. Immunoglobulin superfamily,of which neutralizer is a part, is an expansive and differing gathering of protein involving counter acting agent, MHC class I and II atom, T-cell receptor ,CD3 particle and attachment atoms.

The elements of the individuals from this superfamily run from antigen acknowledgment, attachment atoms to receptor for infections and cytokinesAn neutralizer particle comprises of two indistinguishable light chains and two indistinguishable overwhelming chains, which are connected by disulfide bonds. Every substantial chain has an amino-terminal variable area took after by a steady locale. The substantial chain isotype decides the class of a counter acting agent (IgM; _, IgG; _, IgD; _, IgA; and _, IgE). The five counter acting agent classes have diverse effector capacities, normal serum focuses, and half-lives. Each of the spaces in the immunoglobulin particle has a trademark tertiary structure called the immunoglobulin crease. Inside the amino-terminal variable space of every substantial and light chain are three complementarity-deciding areas (CDRs). These polypeptide districts contribute the antigen-restricting site of an immune response, deciding its specificity. Immunoglobulins are communicated in two structures: discharged neutralizer that is delivered by plasma cells, and layer bound counter acting agent that partners with Ig-_/Ig-_heterodimers to shape the B-cell antigen receptor show on the surface of B cells. The three noteworthy effector works that empower antibodies to expel antigens and murder pathogens are: opsonization, which advances antigen phagocytosis by macrophages and neutrophils; supplement enactment, which initiates a pathway that prompts the age of an accumulation of proteins that can puncture cell films; and neutralizer subordinate cell-intervened cytotoxicity (ADCC), which can execute counter acting agent bound target cells.

5.10SELF ASSESSMENT QUESTION

Multiple Choice Questions

1. Tears contain …

(a) IgA (b) IgG.

(c) Lysozyme. (d) all of the above

2. IgE **…**

(a)is bound together by J chain. (b)binds to mast cells through its Fabregion.

(c)differs from IgG antibody because of its different H chains.

(d)is present in high concentration in serum

3. The Fc region of antibody …

(a)Contains both heavy and light chains. (b)is required for antigen binding.

(c)Is not a requirement for placental transmission.

(d)Generally confers biological activity on the various molecules

4. The fixation of complement by an antigen-antibody reaction can lead to **…**

(a)Formation of a factor chemotactic for mononuclear cells.

(b)Enhanced phagocytosis. (c)Activation of T cells.

(d)Increased synthesis of antibody

5. Ig heavy chains are …

(a)Encoded by a Constant region exon, Variable exon, Diversity exon, and joining exon.

(b)Not glycosylated (c)not important to binding ofantigen.

(d)Expressed by T cells.

Answers:

1. All of the above

2. Differs from IgG antibody because of its different H chains

3 generally confers biological activity on the various molecules

4. Activation of T cells

5. Encoded by a Constant region exon, Variable exon, Diversity exon, and joining exon

Fill in the blanks:

1. Dendritic cells are characterized by their interface between the _____ and _____ immune systems.

2. _____ doesn't receive IgM antibodies from the mother through placental transfer.

3. _____ are antigenic determinants which segregate within a species.

4. The _____ portion of Ig contains the idiotype of the Ig.

5. _____ deficiency can be detected by serum protein electrophoresis.

Answer:

1.Innate & adaptive2. Newborn

3. Allotyoes4. Fab

5. Immunoglobulin

Short Answer Type Questions

1. Define an Epitope?

Ans. The smallest unit of antigenicity is known as epitope.

2. Define Isoantigen?

Ans. Antigens found in some but not all members of a species are called as isoantigen.

3. Define an immunogen?

Ans. An immunogen is a substance that can induces an immune response but which does not necessarily bind to its specific antibody.
Lk b

4. Name the different types of antibody?

Ans. IgG, IgA, IgM, IgD, IgE

5. Define adjuvant?

Ans. The substance, which enhance the immunogenicity of an antigen.

Long Answers Type Questions

1. Explain antigen & its properties of antigenecity?

2. Describe immunoglogulin & its types with their functions?

3. Explain complement system briefy?

4. What is classical pathway explain?

5. Elaborate mode of action of adjuvant?

5.11GLOSSARY

Active immunization: Stimulation of an immune response by exposure to an antigen

adjuvant: Any substance which nonspecifically enhances the immune response to antigen

Agglutination: The "clumping" of a particulate antigen resulting from antibody cross-linking, which may be visible by the naked eye or under a microscope.

Alloantibody: Antibody produced in one organism directed against ALLOTYPE determinants of a genetically different individual of the same species.

Allotype: A genetic POLYMORPHISM or "allelic type" of immunoglobulin

Antiboby: Immunoglobulins (Ig) produced by lymphoid cells of vertebrates with the ability to specifically bind to antigen

Antigen: A molecule which is specifically recognized and can be bound by antibody.

Antigenic determinant: A cluster of epitopes

Autoimmunity: Specific immunity, either humoral or cell-mediated, to constituents of the body's own tissues (autoantigens).

Classical pathway (of complement activation)**:** Activation pathway involving complement components

Complement: An enzymatic complex of serum proteins that is activated by many antigen-antibody reactions

Domain: a structural element of a polypeptide

Epitope: The minimal portion of an antigen molecule which is recongized and bound by an antibody; also referred to as an ANTIGENIC DETERMINANT

Fab: "Antigen-binding" fragment of immunoglobulin molecules produced by proteolysis

Fc: "Crystallizable" fragment of immunoglobulin molecules produced by proteolysis

Fv: The variable region fragment of an antibody heavy or light chain

Hapten: A small molecule which by itself cannot stimulate antibody production but which can be recognized and bound by antibody once the antibody has been formed

Hinge region: That portion of the immunoglobulin heavy chain

Idiotype: The antigenic determinant(s) of an antibody molecule

Immunoassay: Any method utilizing specific antigen-antibody reaction of biological material

Immunogen: Any substance which elicits an immune response.

Immunoglobulin: Member of a family of proteins each made up of light chains and heavy chains linked together by disulfide bonds.

Isotype: In relation to immunoglobulins

J-Chain: "Joining chain"; the polypeptide chain covalently attached to the heavy chain of secreted IgM and polymeric IgA during the process of their polymerization

Membrane attack complex (MAC)**:** Complex of complement components

Monospecific: Describing an antibody population specific for a particular antigenic determinant

Opsonization: The facilitation of phagocytosis by macrophages or granulocytes resulting from antibody and/or complement bound to a target

Passive immunization: Transfer of immunity by transfer of specific antibody

Plasma: The fluid portion of anti-coagulated blood remaining after the "formed elements" (cells and platelets) are removed

Serum: The fluid portion of blood which remains after a blood clot is formed.

5.12 REFERENCES

1. Kind, Thomas J., Goldsby, Richard A., Osborne, Barbara Anne. And Kuby, Janis. (2007): Kuby Immunology. New York: W.H. Freeman.

2.Lydyard,P.M., Whelan, A. and Fanger, M.W., (2004). Immunology. Garland Science/BIOS Scientific Publishers Limited

UNIT: 6 CELL MEDIATED IMMUNITY

CONTENTS

6.1 OBJECTIVES

- To define Cell mediated immunity
- To describe MHC

- To explain exogenous pathway
- To explain endogenous pathway
- To describe functions of T-cells

6.2 MHC (MAJOR HISTOCOMPATIBILITY COMPLEX)

The real histocompatibility complex (MHC) is an arrangement of cell surface proteins fundamental for the gained insusceptible framework to perceive remote atoms in vertebrates, which thusly decides histocompatibility. MHC complex is gathering of qualities on a solitary chromosome that codes the MHC antigens. The significant histocompatibility complex is an accumulation of qualities showed inside a long persistent extend of DNA on chromosome 6 in people. The loci constituting the MHC are profoundly polymorphic; that is, numerous option types of the quality, or alleles, exist at every locus among the populace. In the mammalian genome and, all the more particularly, in the human genome the most factor area known structures the Major Histocompatibility Complex (MHC) that conveys an awesome number of various loci coding for utilitarian qualities. Some of these qualities likewise display numerous variations (alleles), portraying an amazingly polymorphic region.(1) These qualities have a place with the human leukocyte antigen (HLA) framework and code for the significant particles responsible for antigen introduction on the cell surface.

6.3 FUNCTION AND STRUCTURAL ORGANISATION OF MHC

The principle capacity of MHC particles is to tie to peptide sections got from pathogens and show them on the cell surface for acknowledgment by the fitting T-cells. MHC atoms intercede associations of leukocytes, additionally called white platelets (WBCs), which are invulnerable cells, with different leukocytes or with body cells. The MHC decides similarity of benefactors for organ transplant, and in addition one's powerlessness to an immune system malady by means of crosses responding inoculation. The human MHC is likewise called the HLA (human leukocyte antigen) complex. In spite of the fact that the plan of qualities is to some degree unique, as the MHC qualities are composed into locales encoding three classes of atoms.

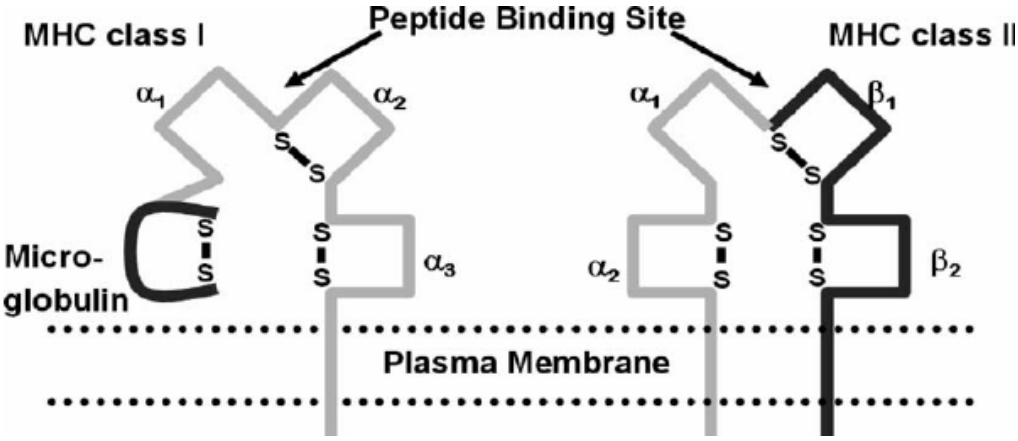


Fig. 6.1 Class of MHC

6.3.1 CLASS I MHC GENES

The structure of MHC class I proteins can be isolated in two globular areas: one locale is situated close to the film and comprises of the b2 microglobulin and a3 spaces. These two areas relate through the four-strand b-sheet yet in a route not quite the same as that in which counter acting agent C spaces pack together. The second district, assist far from the film, comprises of spaces a1 - a2 and contains the antigen restricting site. The structure of a1 and a2 is very comparative and is made out of four antiparallel b-strands took after by a helical locale on one side. The two areas are hydrogen reinforced in a way that the eigth b-strands shape a story over the b2 microglobulin and a3 spaces. The two a-helices shape a hole which is the antigen restricting site. The fissure cans accomodate a 8 deposit (completely broadened) or a 20 buildup (a-helical) peptide. Polymorphic locales in MHC class 1 particles are found both in the floor and also in the helices and characterize which peptides the protein can tie and present to T cells. It encode glycoproteins communicated on the surface of about every single nucleated cell; the significant capacity of the class I quality items is introduction of peptide antigens to TC cells.

6.3.2 CLASS II MHC GENES

MHC class II proteins are polymorphic cell surface proteins which display remote antigens to the T cell receptor of T aide cells. They are heterodimers of two polypeptide chains (a and b) both of which traverse the layer. The a polypeptide comprises of two areas (a1 and a2) which superimpose nearly to the a1 and b2 spaces of MHC class I atoms. The b polypeptide comprises of two spaces (b1 and b2) which superimpose nearly on the a2 and less nearly on

the a3 areas of MHC class I. The a2 and b2 spaces have the crease of an Ig C area and they pack in a route like that seen between the b2 and a3 areas of MHC class I. Contrasts in the helical districts of class II and class I particles clarify why class I atoms tie 8-9 mer peptides though class II tie 12-24 mer peptides. The b2 area of class II atoms associate with the CD4 coreceptor on T-cells. Class I MHC atoms as a rule tie peptides of 8– 10 buildups length (by and large 9-mers, P1– P9) of Figure 3) in a broadened compliance. The cooperation with the MHC protein leaves the side chains of a few deposits of the peptide pointing upward for collaboration with the TCR (see Fig Peptide adaptation in MHC class I and class II proteins). In class II MHC proteins, the notch is open at either end, and the peptide ends are not settled. Consequently, longer successions can be suited contrasted with class I. It encodes glycoproteins communicated basically on antigen-showing cells (macrophages, dendritic cells, and B cells), where they display prepared antigenic peptides to TH cells.

6.3.3 CLASS III MHC GENE

Encode, notwithstanding different items, different emitted proteins that have resistant capacities, including parts of the supplement framework and atoms associated with irritation.

The class I and class II MHC particles have basic highlights and both have parts in antigen handling. By differentiate, the class III MHC district, which is flanked by the class I and II locales, encodes particles that are basic to invulnerable capacity yet have little in the same manner as class I or II atoms. Each arrangement of alleles is alluded to as a haplotype. Decent variety of antigen introduction, interceded by MHC classes I and II, is accomplished in no less than three ways: a life form's MHC collection is polygenic (by means of numerous, cooperating qualities); MHC articulation is co-prevailing (from the two arrangements of acquired alleles); MHC quality variations are very polymorphic (differently fluctuating from life form to creature inside an animal categories). MHC is the tissue-antigen that permits the resistant framework (all the more particularly T cells) to tie to, perceive, and endure itself (autorecognition). MHC is additionally the chaperone for intracellular peptides that are complexed with MHCs and displayed to TCRs as potential outside antigens. MHC interfaces with TCR and its co-receptors to streamline restricting conditions for the TCR-antigen connection, as far as antigen restricting proclivity and specificity, and flag transduction viability

Structure: The MHC complex dwells in the short arm of chromosome 6 and general size of the MHC is roughly 3.5 million base sets. The class I quality complex contains three loci A, B and C, each of which codes of α chain polypeptides. The class II quality complex likewise contains no less than three loci, DP, DQ and DR; each of these loci codes for one α and a variable number of β chain polypeptides. Class III area isn't really a piece of the HLA complex, however is situated inside the HLA district, since its segments are either identified with the elements of HLA antigens or are under comparative control components to the HLA qualities. Class III antigens are related with proteins in serum and other body liquids and have no part in join dismissal. HLA specificities are recognized by a letter for locus and a number (A1, B5, and so forth.), and the haplotypes are distinguished by singular specificities (e.g., A1, B7, Cw4, DP5, DQ10, DR8). Specificities which are characterized by genomic investigation (PCR), are named with a letter for the locus and a four digit number (e.g. A0101, B0701, C0401, and so forth.).

6.4 ANTIGEN PROCESSING & PRESENTATION

Antigen handling is an immunological procedure that gets ready antigens for introduction to unique cells of the resistant framework called T lymphocytes. It is thought to be a phase of antigen introduction pathways. Acknowledgment of remote antigens by a T cell requires peptides got from the antigen be shown inside the parted of a MHC atom on the film of a cell. The arrangement of these peptide-MHC buildings requires that a protein antigen be corrupted into peptides by a succession of occasions called antigen handling. The corrupted peptides at that point connect with MHC atoms inside the cell inside, and the peptide-MHC edifices are transported to the film, where they are shown is named as antigen introduction. CD4_and CD8_T cells can perceive antigen just when it is introduced by a self-MHC particle, a characteristic called self-MHC confinement. An assortment of cells can work as antigen-displaying cells. Their recognizing highlight is their capacity to express class II MHC particles and to convey a co-stimulatory flag. Three cell sorts are named proficient antigen-displaying cells: dendritic cells, macrophages, and B lymphocytes. These cells vary from each other in their components of antigen take-up, in whether they constitutively express class II MHC atoms, and in their co-stimulatory action. Dendritic cells are the best of the antigen-displaying cells. Since these cells constitutively express an abnormal state of class II MHC atoms and co-stimulatory movement, they can initiate credulous TH cells. Macrophages must be initiated by phagocytosis of particulate antigens previously they

express class II MHC atoms or the co-stimulatory B7 layer particle. B cells constitutively express class II MHC atoms yet should be initiated before they express the co-stimulatory B7 particle. A few other cell sorts, delegated nonprofessional antigen-displaying cells, can be incited to express class II MHC particles or a co-stimulatory flag (Table 8-1). Huge numbers of these phones work in antigen introduction just for brief timeframes amid a supported provocative reaction. Antigen handling includes two unmistakable pathways for preparing of antigens to dispose of intracellular and extracellular antigens i.e. endogenous pathway and exogenous pathway.

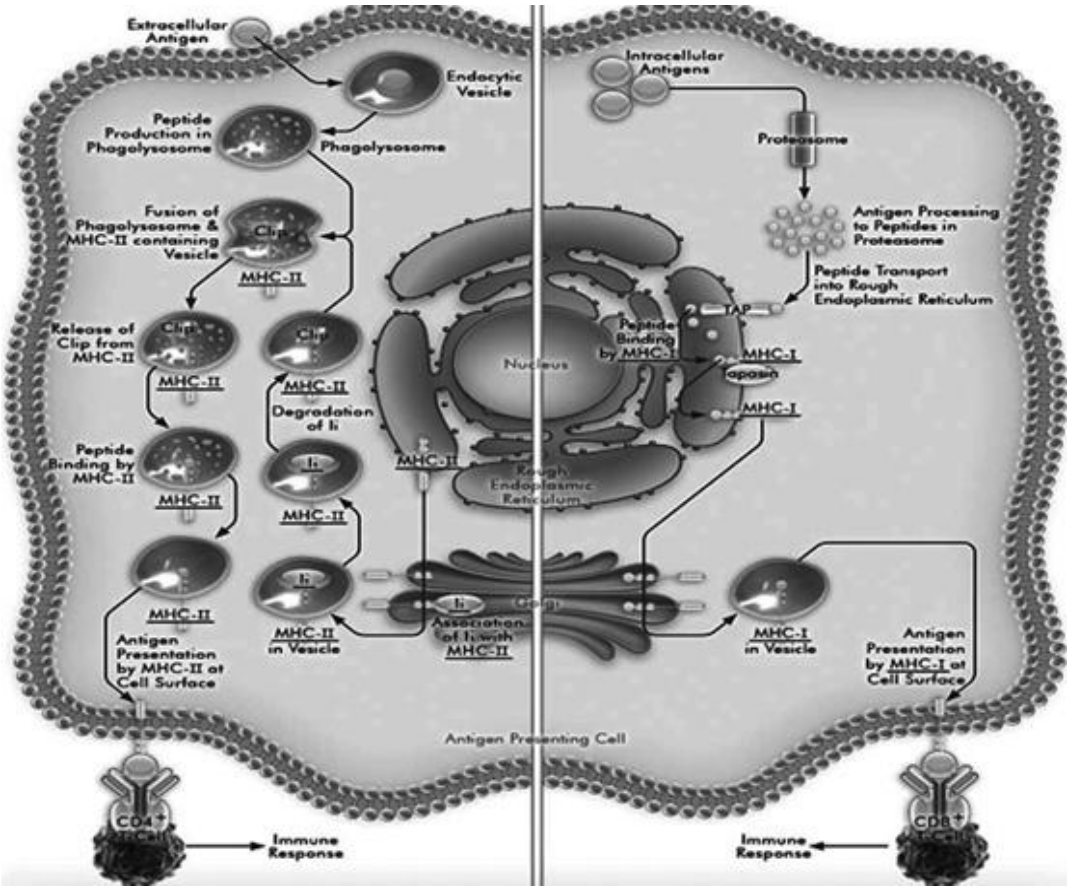


Fig.6.2 Antigen processing

6.4.1 The Endogenous Pathway

Endogenous antigens (those produced inside the cell) are handled in the cytosolic pathway and displayed on the film with class I MHC particles. The pathway by which endogenous antigens are corrupted for introduction with class I MHC atoms uses the same pathways associated with the typical turnover of intracellular proteins. The endogenous pathway is utilized to exhibit cell peptide sections on the cell surface on MHC class I particles. On the

off chance that an infection had tainted the cell, viral peptides would likewise be exhibited, enabling the insusceptible framework to perceive and murder the contaminated cell. Exhausted proteins inside the cell move toward becoming ubiquitinated, stamping them for proteasome debasement. Intracellular proteins are corrupted into short peptides by a cytosolic proteolytic framework introduce in all cells. Those proteins focused for proteolysis regularly have a little protein, called ubiquitin, joined to them. Ubiquitin-protein conjugates can be debased by a multifunctional protease complex called a proteasome. The transporter protein, assigned TAP (for transporter related with antigen preparing) is a layer spreading over heterodimer comprising of two proteins: TAP1 and TAP2. Notwithstanding their various transmembrane sections, the TAP1 and TAP2 proteins each have a space anticipating into the lumen of the RER, and an ATP-restricting area that undertaking into the cytosol. Both TAP1 and TAP2 have a place with the group of ATP-restricting tape proteins found in the films of numerous cells, including microscopic organisms; these proteins intervene ATP-subordinate transport of amino acids, sugars, particles, and peptides. Peptides created in the cytosol by the proteasome are translocated by TAP into the RER by a procedure that requires the hydrolysis of ATP. TAP has the most astounding fondness for peptides containing 8– 10 amino acids, which is the ideal peptide length for class I MHC authoritative. Moreover, TAP support peptides with hydrophobic or fundamental carboxyl-terminal amino acids, the favored stay deposits for class I MHC particles. In this manner, TAP is upgraded to transport peptides that will cooperate with class I MHC particles. Once the peptide is transported into the ER lumen it ties to the split of the anticipating MHC class I particle, settling the MHC and enabling it to be transported to the cell surface by the golgi mechanical assembly.

6.4.2 THE EXOGENOUS PATHWAY

Exogenous antigens (those taken up by endocytosis) are handled in the endocytic pathway and displayed on the layer with class II MHC atoms. The exogenous pathway is used by specific antigen exhibiting cells to display peptides got from proteins that the cell has endocytosed. The peptides are exhibited on MHC class II particles. Proteins are endocytosed and corrupted by corrosive ward proteases in endosomes. B cells, disguise antigen successfully by receptor-intervened endocytosis utilizing antigen-particular film counter acting agent as the receptor. Once an antigen is disguised, it is corrupted into peptides inside compartments of the endocytic preparing pathway. The endocytic pathway includes three progressively acidic compartments early endosomes, late endosomes, or endolysosomes and

lysosomes. Disguised antigen moves from ahead of schedule to late endosomes lastly to lysosomes, experiencing hydrolytic proteins and a lower pH in every compartment. Inside the compartments of the endocytic pathway, antigen is debased into oligopeptides of around 13–18 residues,which tie to class II MHC atoms. The beginning MHC class II protein in the harsh ER has its peptide-restricting separated obstructed by Ii (the invariant chain; a trimer) to keep it from restricting cell peptides or peptides from the endogenous pathway. The invariant chain likewise encourages MHC class II's fare from the ER in a vesicle. This breakers with a late endosome containing the endocytosed, corrupted proteins. The invariant fasten is then softened up stages, leaving just a little section called "Class II-related invariant chain peptide" (CLIP) which still obstructs the peptide restricting parted. A MHC class II-like structure, HLA-DM, expels CLIP and replaces it with a peptide from the endosome. The stable MHC class-II is then displayed on the cell surface.

6.5 FUNCTIONS OF T-CELLS

A T cell, or T lymphocyte, is a sort of lymphocyte (a subtype of white platelet) that assumes a focal part in cell-intervened resistance. They are called T cells since they develop in the thymus from thymocytes. The few subsets of T cells each have an unmistakable capacity. The lion's share of human T cells modify their alpha and beta chains on the cell receptor and are named alpha beta T cells (αβ T cells) and are a piece of the versatile resistant framework. Lymphocytes perceive a "non-self" target, for example, a pathogen, simply after antigens have been prepared and exhibited in blend with a "self" receptor, called a noteworthy histocompatibility complex (MHC) atom. There are two noteworthy subtypes of T cells: the executioner T cell, which slaughters cells that are tainted with infections (and different pathogens) or are generally harmed or broken, and the assistant T cell, which directs both natural and versatile resistant reactions and figures out which safe reactions the body makes to a specific pathogen. These cells have no cytotoxic action and don't murder tainted cells or clear pathogens straightforwardly. White blood cells can be isolated into three noteworthy gatherings in light of capacity: cytotoxic T cells, partner T cells (Th), and administrative T cells (Tregs).

6.5.1 CYTOTOXIC T CELLS

Cytotoxic T lymphocytes will be lymphocytes that slaughter other ("target") cells. Target cell incorporates infection tainted cells; cells contaminated with intracellular bacterial or

protozoal parasites; allografts, for example, transplanted kidney, heart, lungs and malignancy cells. CTL have a place with the CD8+ subset of T cells; utilize the αβ T-cell receptor for antigen (TCR) in this manner perceive antigens settled ready of class I histocompatibility (MHC) atoms. On the off chance that they experience the antigen/MHC for which their TCR is particular, they enter the cell push and experience a few rounds of mitosis took after by separation into effector ("executioner") cells. Their separation incorporates framing countless lysosomes loaded down with proteins: perforin and a few sorts of granzyme. They are helped in these exercises by aide T cells that emit stimulatory cytokines like IL-21. A large portion of CTLs kick the bucket of apoptosis when they have done their activity, yet some progress toward becoming memory cells which are seemingly perpetual cells ready to react to the antigen in the event that it ought to return.

6.5.2 T - ASSISTANT CELL

The T aide cells (Th cells) are a sort of T cell that assumes an imperative part in the invulnerable framework, especially in the versatile safe framework. They help the movement of other safe cells by discharging T cell cytokines. These cells help smother or direct invulnerable reactions. They are fundamental in B cell counter acting agent class exchanging, in the actuation and development of cytotoxic T cells, and in boosting bactericidal movement of phagocytes, for example, macrophages. The resistant framework must accomplish an adjust of affectability so as to react to remote antigens without reacting to the antigens of the host itself. At the point when the insusceptible framework reacts to low levels of antigen that it as a rule shouldn't react to, an extreme touchiness reaction happens. Excessive touchiness is accepted to be the reason for sensitivity and some auto-resistant sickness. Extreme touchiness responses is isolated into four sorts

- **Type 1** excessive touchiness incorporates normal invulnerable issue, for example, asthma, hypersensitive rhinitis (feed fever), skin inflammation, urticaria (hives) and hypersensitivity. These responses all include IgE antibodies, which require a Th2 reaction amid partner T cell advancement. Preventive medications, for example, corticosteroids and montelukast, concentrate on smothering pole cells or other unfavorably susceptible cells; T cells don't assume an essential part amid the genuine fiery reaction. It's imperative to take note of that the numeral designation of extreme touchiness "sorts" does not associate (and is totally random) to the "reaction" in the Th demonstrate.

- Type 2 and Type 3 touchiness both include complexities from auto-safe or low proclivity antibodies. In both of these responses, T cells may assume an assistant part in producing these auto-particular antibodies, albeit some of these responses under Type 2 extreme touchiness would be viewed as typical in a solid safe framework (for instance, Rhesus factor responses amid labor is an ordinary invulnerable reaction against youngster antigens). The comprehension of the part of assistant T cells in these reactions is restricted however it is for the most part believed that Th2 cytokines would advance such issue. For instance, examines have proposed that lupus (SLE) and other auto-safe maladies of comparable nature can be connected to the generation of Th2 cytokines.
- Type 4 excessive touchiness, otherwise called postponed sort extreme touchiness, are caused by means of the over-incitement of insusceptible cells, usually lymphocytes and macrophages, bringing about constant irritation and cytokine discharge. Antibodies don't assume an immediate part in this hypersensitivity sort. Immune system microorganisms assume an imperative part in this extreme touchiness, as they initiate against the boost itself and advance the actuation of different cells; especially macrophages through Th1 cytokines.

6.5.3 REGULATORY T CELL

The administrative T cells otherwise called silencer T cells, are a subpopulation of T cells which tweak the resistant framework, keep up resilience to self-antigens, and anticipate immune system infection. Tregs are immunosuppressive and by and large smother or down-control enlistment and expansion of effector T cells. Tregs can treat immune system malady and malignancy and can encourage organ transplantation. T administrative cells are a segment of the resistant framework that smother insusceptible reactions of different cells. This is an essential "self-check" incorporated with the invulnerable framework to avert exorbitant responses. Administrative T cells are associated with closing down safe reactions after they have effectively wiped out attacking creatures, and furthermore in forestalling autoimmunity. The invulnerable framework must have the capacity to separate amongst self and non-self. Whenever self/non-self separation comes up short, the resistant framework decimates cells and tissues of the body and accordingly causes immune system sicknesses. Administrative T cells effectively stifle actuation of the insusceptible framework and forestall obsessive self-reactivity, i.e. immune system ailment. The basic part administrative T cells

play inside the invulnerable framework is confirm by the serious immune system disorder that outcomes from a hereditary insufficiency in administrative T cells. control component is through the IL-2 criticism circle. Antigen-initiated T cells deliver IL-2 which at that point follows up on IL-2 receptors on administrative T cells cautioning them to the way that high T cell movement is happening in the locale, and they mount a suppressory reaction againt them.

6.6 SUMMARY

The capacity of MHC particles is to tie peptide pieces got from pathogens and show them on the cell surface for acknowledgment by the proper T cells. The outcomes are quite often malicious to the pathogen-infection contaminated cells are slaughtered, macrophages are enacted to eliminate microscopic organisms living in their intracellular vesicles, and B cells are initiated to deliver antibodies that dispose of or kill extracellular pathogens. In this manner, there is solid specific weight for any pathogen that has transformed such that it escapes introduction by a MHC particle. Two separate properties of the MHC make it troublesome for pathogens to sidestep insusceptible reactions along these lines. To start with, the MHC is polygenic: it contains a few distinctive MHC class I and MHC class II qualities, so every individual has an arrangement of MHC atoms with various scopes of peptide-restricting specificities. Second, the MHC is exceedingly polymorphic; that is, there are different variations of every quality inside the populace all in all. The MHC qualities are, truth be told, the most polymorphic qualities known. In this area, we will depict the association of the qualities in the MHC and talk about how the variety in MHC particles emerges. We will likewise perceive how the impact of polygeny and polymorphism on the scope of peptides that can be bound adds to the capacity of the resistant framework to react to the large number of various and quickly advancing pathogens. • B-cell and discharged antibodies perceive and tie solvent antigen while T-cell perceive and react to antigenic peptide showed on MHC. Antigen either exogenous or endogenous are first prepared and after that displayed on MHC to invigorate T cells i.e. TH cell and Tcyt cell. Cells that show peptides related with class I MHC atoms to CD8+ Tcyt cell is alluded to as target cell. Cell that showcases peptides related class II MHC particle to TH cell is called as antigen-exhibiting cell. There are three sorts of antigen-showing cells - dendritic cells, macrophages, B-lymphocytes called as expert antigen-introducing cells. Non-proficient antigen-introducing cell express class-II MHC atom upon incitement by interferon. There are two primary preparing pathways prompting either class-I or class-II MHC particles. One is cytosolic

pathway utilized by endogenous antigen prompting class I MHC particles. In the second pathway, exogenous antigens are endocytosed, handled and displayed on class-II MHC atoms. Endogenous antigens are first labeled with ubiquitin, and afterward hacked by cell corruption chamber-proteasome into 8-10 amino corrosive deposit peptides. The handled peptides at that point tie peptide transporter TAP1 and TAP2 confined in ER and enter ER. Peptides tie and settle an and b chains of class I MHC atoms. Stacked and completely collapsed stable class I MHC-peptide complex travel through Golgi to the surface of the cell and appeared to Tcyt cell reconnaissance. Introduction and handling of exogenous antigens begins with endocytosis/phagocytosis of antigen from extracellular milieu. Endosome shaped circuits with lysosome bringing about the debasement of protein antigens. Lysosome, which contains different cluster of hydrolases process the protein antigens to produce short peptides. In RER, class II MHC atoms are amassed and after that peptide restricting score is hindered by invariant chain(li). The invariant chain coordinates vesicle containing class II MHC particles towards endosome containing antigenic peptides. When class II MHC – Ii complex experiences antigenic peptide, Ii-tie is first divided to frame CLIP which at that point separates. Expulsion of CLIP and stacking of class II MHC particles with antigenic peptide is catalyzed by non-established class II MHC atom HLA-DM. Peptide-class II MHC atom is then exchanged to cell surface for T-cell investigation. Non-peptide antigens, for example, lipid, glycolipid antigen are displayed on non-traditional class I atom – CD1 while prenylpyrophosphates and alkylamines are introduced on novel, newfound antigen showing particles. White blood cells begin as foundational microorganisms (early sorts of cells that have not yet completely developed) and are created by bone marrow. To develop, these undeveloped cells move to the thymus, where they can remain for up to three weeks. Around 99% of T cells don't make it to development. This is on account of the body is exceptionally particular about what T cells are created with the goal that they don't make harm the body's own cells. In the thymus, the T cells are given T cell receptors, of which there are a few sorts. The sort of receptor got figures out what kind of T cell it will be, what its part is, and which cell it can collaborate with. White blood cells work both through the arrival of substances into the blood, and by flagging B cells through contact. Motioning for development and initiation of B cells.Activation of cells that can 'eat' outside substances. Incitement of cytotoxic T cells amid a viral contamination. Flagging development in cells, including other T cells, macrophages and eosinophils.

6.7 GLOSSARY

Acute phase proteins. Found in the blood soon after the onset of an infection, they limit damage caused by the organism and implement repair

ADCC. Antibody dependent cellular cytotoxicity

Adhesion molecules. Cell surface molecules that are involved in cell to cell interactions

Affinity. The binding strength of a single receptor to its ligand

Allelic exclusion. In a heterozygous individual only one of the two allelic forms

Anergy. A state of tolerance involving non-responsiveness to antigen rather than cell deletion

Antigen presentation. The display of peptide fragments bound to MHC molecules on the cell surface, necessary for recognition by T cells.

Antigen processing. Enzymatic degradation of proteins into peptides to be associated with MHC molecules for T cell recognition

Antigen-presenting cell (apc): A cell capable of presenting antigenic peptides associated with MHC Class II to a T-cell

Clonal selection. Antigen selects specific B or T cells to expand into clones

Complementarity determining regions (CDR): The hypervariable amino acid sequences within antibody and T-cell receptor variable regions which interact with complementary amino acids on the antigen or peptide–MHC complex

Cross-matching. Used to test whether recipients have preformed antibodies to blood group or histocompatibility antigens (HLA) to donor tissues that could interfere with successful transplantation

H-2: The major histocompatibility complex (MHC) of the mouse

Haplotype. A linked set of genes associated with one haploid genome

HLA. Human leukocyte antigens are the major histocompatibility antigens in man that bind peptides and present them to T cells

Integrins. One of the ‘families’ of adhesion molecules

IR genes. Immune response genes are genetic polymporphisms that control immune responses; they include the HLA genes

Lamina propria: The connective tissue underlying the epithelium at mucosal sites

Locus: The position in a chromosome at which a particular gene is located

Membrane attack complex: The terminal complement components c5b, C6, C7, C8, C9 that result in pore formation and membrane damage.

MHC restriction: The necessity that T-cells recognize processed antigen only when presented by MHC molecules of the original haplotype associated with T-cell priming.

MHC: Major histocompatibility complex; the genetic locus that codes for HLA

Mixed lymphocyte reaction (MLR): A T-cell proliferative response induced by cells expressing allogeneic MHC.

Pinocytosis: Internalization of soluble extracellular material by formation of small membrane vesicles

Polymorphism. Genetic polymorphism is where a gene has several allelic forms present at a single gene locus

Qa antigens: 'Non-classical' MHC class I molecules of mice.

Serology. The use of antibodies to detect and measure antigens

6.8 SELF ASSESSMENT QUESTION

Fill in the blanks:

1. _____activate large numbers of T cells by directly binding to the TCRβ chain and class II MH**C**
2. A_____ isthe total set of MHC alleles present on each chromosome.
3. _____ is not induced by antihistamines.
4. Tumor immune surveillance is mediated by _____.
5. Helper T cells are distinguished from cytotoxic T cells by the presence of _____.

Answer:

1. Superantigens
2. HLA haplotype
3. Immunosuppression
4. NK cells
5. CD4

Multiple Choice Questions

1. TCR gene rearrangement

A. Takes place primarily in the bone marrow. B. is antigen independent.

C. involves immunoglobulin.

D requires co-stimulation by antigen presenting cells.

2. The class I MHC processing pathway primarily

A. processes antigens that are present in the cytosol.

B. processes antigens from the extracellular environment.

C. generates peptides, complexes them with class I MHC molecules for presentation to helper T cells.

D. generates peptides, complexes them with class I MHC molecules for presentation to NK cells.

3. Host antibody against a tumor would most likely be directed against

A. MHC class II antigens. B. viral antigens.

C. differentiation antigens. D. MHC class I antigens

4. Major histocompatibility antigens are not …

A. linked with a number of autoimmune diseases.

B. important for interactions between T and B cells during an immune response.

C. the only antigens which result in graft rejection.

D. important for graft versus host reactions

5. HLA disease association …

A. means that the particular HLA antigen or

Haplotype involved causes the disease.

B. may in some instances be useful in diagnosis.

C. means that every person with that HLA type will contract the disease.

D. may suggest that genes near the MHC locus code for T cell antigen receptors specific for self antigens

Answers:

1.is antigen independent 2. Processes antigens that are present in the cytosol

3. Viral antigens 4. The only antigens which result in graft rejection

5. May in some instances be useful in diagnosis?

Short Answer Type Questions

1. Define MHC?

Ans. Major Histocompatibility complex

2. Name the pathways included for antigen presentation?

Ans. Exogenous pathway & Endogenous pathway

3. Name the types of T-cells?

Ans. T-helper cells, Regulatory T cell, Cytotoxic T cells

4. Define HLA?

Ans. The human leukocyte antigen (HLA) system or complex is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans.

5. Define hypersensitivity?

Ans. Hypersensitivity also called hypersensitivity reaction is a set of undesirable reactions produced by the normal immune system, including allergies and autoimmunity.

Long Answers Type Questions

1. Explain MHC structure in brief?

2. Describe Antigen processing & presentation in detail?

3. Discuss endogenous & exogenous pathway in brief?

4. Elaborate functions of T-cells?

UNIT: 7 APPLICATIONS OF IMMUNOLOGY

CONTENTS

7.1 OBJECTIVES

- To define vaccines & their types
- To understand immunodiagnostics & its applications
- To describe the methods of agglutination
- To explain the principle of Complement Fixation Test
- To describe immunotherapy & its various forms

7.1.1 VACCINES

The expression "vaccine" was authored by **Louis Pasteur** to honor first fruitful vaccination against little pox by Edward Jenner. The term vaccine was gotten from "vacca", which means bovine, since Edward Jenner utilized cowpox infection (Vaccinia) to anticipate smallpox contamination. Invulnerability can be gained either by normal procedures (more often than not by exchange from mother to baby or by past disease by the life form) or by fake means, for example, infusion of antibodies or immunizations. An antibody is an organic planning that gives dynamic procured resistance to a specific illness. An immunization regularly contains an operator that looks like a malady causing microorganism and is frequently produced using debilitated or murdered types of the organism, its poisons or one of its surface proteins. The operator empowers the body's resistant framework to perceive the specialist as a risk, wreck it, and keep a record of it with the goal that the safe framework would more be able to effectively perceive and pulverize any of these microorganisms that it later experiences. The organization of antibodies is called immunization. Immunization is the best technique for anticipating irresistible infections. Inoculation includes think presentation to antigen under conditions where sickness ought not come about. Inoculation is gone for initiating dynamic insusceptibility in an individual, so consequent contact with the microorganism following regular disease prompts solid defensive invulnerable reaction. The defensive invulnerability may include discharge of killing antibodies or generation of memory CTL or Th1 cells. An antibody is a suspension of entire (live or inactivated) or fractionated microscopic organisms or infections that have been rendered non-pathogenic, and is given to instigate an insusceptible reaction and anticipate sickness. Despite the fact that no immunization is totally protected or totally powerful, their utilization is firmly upheld by their advantage to-chance proportion. The antibody vial may contain applicable antigen,

adjuvant (typically alum), additives and additionally hints of protein got from the phones in which the immunization operator was refined. Adjuvants generally are utilized to help resistant reaction, especially for more established individuals (50– 75 years and up), whose safe reaction to a straightforward antibody may have debilitated. In the event that an inoculated individual develops the sickness immunized against, the ailment is probably going to be less destructive than in unvaccinated casualties. The resistant framework perceives antibody operators as remote, demolishes them, and "recalls that" them. At the point when the harmful rendition of a specialist is experienced, the body perceives the protein coat on the infection, and along these lines is set up to react, by killing the objective operator before it can enter cells, and perceiving and obliterating contaminated cells previously that operator can increase to tremendous numbers. The specialists utilized for inciting detached resistance incorporate antibodies from people or creatures, while dynamic vaccination is accomplished by immunization with microbial pathogens that initiate insusceptibility however don't cause illness or with antigenic segments from the pathogens. A monovalent antibody is intended to inoculate against a solitary antigen or single microorganism. A multivalent or polyvalent immunization is intended to inoculate against at least two strains of a similar microorganism, or against at least two microorganisms.

Fig 7.1 Vccines

Immunization is done through different methods, most ordinarily immunization. Immunizations against microorganisms that reason illnesses can set up the body's resistant

framework, in this way battling or keep a contamination. The most vital components of the insusceptible framework that are enhanced by vaccination are the T cells, B cells, and the antibodies B cells create. Memory B cells and memory T cells are in charge of a quick reaction to a moment experience with an outside particle. Insusceptibility to irresistible microorganisms can be accomplished by dynamic or inactive vaccination. Inoculation is accomplished in a dynamic or aloof way; immunization is a dynamic type of vaccination.

7.1.2 ACTIVE IMMUNIZATION

The objective of dynamic inoculation is to inspire defensive invulnerability and immunologic memory. At the point when dynamic vaccination is fruitful, an ensuing presentation to the pathogenic specialist inspires an increased insusceptible reaction that effectively takes out the pathogen or forestalls sickness interceded by its items. Dynamic inoculation can happen normally when a man interacts with, an organism. The safe framework will in the long run make antibodies and different resistances against the organism. Whenever, the resistant reaction against this organism can be exceptionally effective. Dynamic vaccination can be accomplished by normal contamination with a microorganism, or it can be obtained falsely by organization of an immunization. In dynamic inoculation, as the name suggests, the resistant framework assumes a dynamic part—multiplication of antigen-responsive T and B cells brings about the arrangement of memory cells. Manufactured dynamic inoculation is the place the organism, or parts of it, are infused into the individual before they can take it in normally. On the off chance that entire organisms are utilized, they are pre-treated.

7.1.3 PASSIVE INOCULATION

Passive vaccination is the place pre-blended components of the insusceptible framework are exchanged to a man with the goal that the body does not have to create these components itself. In detached inoculation, preformed antibodies are exchanged to a beneficiary, happens normally by exchange of maternal antibodies over the placenta to the creating embryo. This technique for vaccination starts to work rapidly, yet it is short enduring, on the grounds that the antibodies are normally separated, and if there are no B cells to create more antibodies, they will vanish. Manufactured uninvolved vaccination is regularly regulated by infusion and is utilized if there has been a current flare-up of a specific ailment or as a crisis treatment.

Vaccines are dead or inactivated creatures or cleansed items got from them. There are a few sorts of antibodies being used. These speak to various procedures used to attempt to diminish the danger of sickness while holding the capacity to incite an advantageous invulnerable reaction.

7.2 TYPES OF VACCINES

A. Killed/Inactivated Vaccines: When it is perilous to utilize live microorganisms to get ready antibodies, they are inactivated. These are arrangements of the ordinary (wild sort) irresistible, pathogenic microorganisms that have been rendered non-pathogenic, as a rule by treatment with utilizing warmth, formaldehyde or gamma illumination so they can't repeat by any stretch of the imagination. Such executed antibodies change incredibly in their adequacy. A few antibodies contain inactivated, however already harmful, small scale living beings that have been pulverized with chemicals, warmth, radiation, or anti-microbials.

Exp.: flu, bubonic torment, cholera, hepatitis A, polio and rabies

Advantages:

- Safe to utilize and can be given to resistant lacking and pregnant people.
- Cheaper than live constricted antibody
- Storage not as basic as live antibody

Disadvantages:

- Since the microorganisms can't increase, an expansive number are required to fortify invulnerability.
- Periodic promoters must be given to look after insusceptibility.
- Only humoral resistance can be initiated.
- Most slaughtered antibodies must be infused.
- Anaphylactic excessive touchiness to eggs may happen in beneficiaries of flu antibody.
- Inactivation, for example, by formaldehyde on account of the Salk antibody, may modify antigenicity.
- Presence of some un-inactivated organisms can prompt immunization related ailment

B. Live Attenuated Vaccine: These immunizations are made out of live, weakened microorganisms that reason a constrained contamination in their hosts adequate to instigate an insusceptible reaction, however deficient to cause sickness. To make a

weakened immunization, the pathogen is developed in outside host, for example, creatures, embryonated eggs or tissue culture, under conditions that make it less destructive. A few antibodies contain live, lessened microorganisms. A considerable lot of these are dynamic infections that have been developed under conditions that impair their destructive properties, or that utilization firmly related however less risky living beings to deliver a wide resistant reaction. Albeit most weakened antibodies are viral, some are bacterial in nature. The strains are changed to a non-pathogenic frame. A few mutants will be chosen that have a superior capacity to develop in the remote host. These have a tendency to be less harmful for the first host. These antibodies might be given by infusion or by the oral course. A noteworthy preferred standpoint of live infection antibodies is that since they cause disease, the immunization nearly imitates the characteristic boost to the resistant framework.

Exp.: incorporate the viral illnesses yellow fever, measles, rubella, and mumps, and the bacterial ailment typhoid.

Advantages:

- Infectious organisms can animate age of memory cell and additionally humoral invulnerable reactions.
- Since these can duplicate in the host, less amounts must be infused to instigate security.
- A single organization of antibody frequently has a high viability in delivering enduring insusceptibility. Numerous supporter measurements isn't required.
- Whole organisms empower reaction to antigens in their normal adaptation. They raise safe reaction to every single defensive antigen.
- Some live antibodies are given orally; such immunizations prompt mucosal insusceptibility and IgA union, which gives more assurance at the ordinary site of section.
- Oral arrangements are more affordable than giving infusions.
- They prompt end of wild sort infection from the group

Disadvantages:

- These once in a while return to its destructive shape and cause sickness.
- Live immunizations can't be offered securely to resistant smothered people. Organization of live weakened antibodies to individuals with hindered insusceptible capacity can cause genuine sickness or demise in the immunization beneficiary.

- Since they are live and in light of the fact that their action relies upon their suitability, appropriate capacity is basic

C. **Subunit vaccines:** Presenting an inactivated or constricted miniaturized scale creature to a safe framework, a section of it can make an invulnerable reaction Subunit immunizations contain refined antigens rather than entire life forms. Such a planning comprises of just those antigens that evoke defensive resistance. Subunit antibodies are made out of toxoids, subcellular sections, or surface antigens. Organization of entire life form, as if there should arise an occurrence of pertussis was discovered ominous resistant responses bringing about extreme reactions. The viability of subunit antibodies in expanded by giving them in adjuvants. Adjuvants moderate antigen discharge for a more maintained invulnerable incitement.

   Exp. incorporate the subunit antibody against Hepatitis B infection, the infection like molecule (VLP) antibody against human papillomavirus (HPV) that is made out of the viral real capsid protein, the hemagglutinin and neuraminidase subunits of the flu infection and Subunit immunization for torment inoculation.

   Advantages:

- These are sheltered to invulnerable smothered individuals
- They are less inclined to initiate reactions.

   Disadvantages:

- Antigens may not hold their local adaptation, with the goal that antibodies delivered against the subunit may not perceive a similar protein on the pathogen surface.
- Isolated protein does not empower the insusceptible framework and in addition an entire living being immunization.

D. **Peptide vaccines:** A peptide antibody is any peptide which serves to vaccinate a creature against a pathogen. Peptide immunizations are frequently manufactured and impersonate normally happening proteins from pathogens. Peptide immunization comprises of those peptides from the microbial antigen that empowers defensive insusceptibility. Manufactured peptides are delivered via computerized machines instead of by microorganisms. Peptide immunogenicity can be expanded by giving them in ISCOMS, lipid micelles that vehicle the peptides specifically into the cytoplasm of dendritic cells for introduction on Class I MHC. Infused peptides, which are substantially littler than the first infection protein, prompt an IgG reaction.

Example: spf66 anti-malarial vaccine

Advantages:

- If the peptide that instigates defensive resistance is recognized, it can be integrated effortlessly on a vast scale.
- It is sheltered and can be directed to resistant inadequate and pregnant people.

Disadvantage:

- Poor antigenicity. Peptide pieces don't invigorate the insusceptible framework and in addition an entire life form immunization.
- Since peptides are nearly connected with HLA alleles, a few peptides may not be all around successful at instigating defensive resistance.

E. Recombinant Vaccines: The antibodies are delivered utilizing recombinant DNA innovation or hereditary building. Recombinant antibodies are those in which qualities for wanted antigens of an organism are embedded into a vector. Distinctive systems utilized as a part of arrangement are:

- Using the designed vector (e.g., Vaccinia infection) that is communicating wanted antigen as an immunization
- The designed vector (e.g., yeast) is made to express the antigen, such is vector is developed and the antigen is sanitized and infused as a subunit antibody. Other articulation vectors incorporate the microbes Escherichia coli, mutant Salmonella spp., and BCG.
- Introduction of a change by erasing a segment of DNA with the end goal that they are probably not going to return can make a lessened live immunization.
- Live constricted antibodies can likewise be created by reassortment of genomes of destructive and avirulent strains.
- Genes coding for noteworthy antigens are brought into plants, with the end goal that the natural products created bear outside antigens.

Examples:

- Hepatitis B Virus (HBV) antibody is a recombinant subunit immunization. Hepatitis B surface antigen is created from a quality transfected into yeast (Saccharomyces cerevisiae) cells and cleansed for infusion.
- Vaccinia infection might be designed to express protein antigens of HIV, rabies and so on. Remote qualities cloned into the viral genome are communicated on the surface of tainted cells in relationship with class I MHC particles.

- The antigen-MHC complex instigates a Tc cell reaction.
- B subunit of cholera poison, the B subunit of warmth labile E. coli enterotoxin (LT), and one of the glycoprotein film antigens of the malarial parasite are being created utilizing this strategy.
- Salmonella typhimurium built to express antigens of Vibrio cholerae.
- Bacille Calmette-Guérin immunization strain designed to express qualities of HIV-1.
- Reassortment of genomes amongst human and avian strains to make Influenza immunization. Human and swine strains to make Rotavirus antibody

Advantages:

- Those vectors that are sheltered as well as simple to develop and store can be picked.
- Antigens which don't inspire defensive invulnerability or which evoke harming reactions can be disposed of from the immunization. Case Cholera poison A can be securely expelled from cholera poison.

Disadvantages:

- Since the qualities for the coveted antigens must be found, cloned, and communicated effectively in the new vector, the cost of generation is high.
- When designed vaccinia infection is utilized to inoculate, mind must be taken to save safe lacking people

F. DNA Vaccines: Like recombinant antibodies, qualities for the coveted antigens are found and cloned. The DNA is infused into the muscle of the creature being immunized, as a rule with a "quality firearm" that utilizations compacted gas to blow the DNA into the muscle cells. DNA can be brought into tissues by assaulting the skin with DNA-covered gold particles. It is likewise conceivable to bring DNA into nasal tissue in nose drops. Some muscle cells express the pathogen DNA to invigorate the invulnerable framework. DNA immunizations have actuated both humoral and cell resistance. These antibodies are still in trial arrange.

Advantages:

- DNA is exceptionally steady, it opposes extraordinary temperature and subsequently stockpiling and transport are simple.
- A DNA grouping can be changed effortlessly in the research facility.
- The embedded DNA does not imitate and encodes just the proteins of intrigue
- There is no protein part thus there will be no safe reaction against the vector itself.

- Because of the way the antigen is exhibited, there is a cell-interceded reaction that might be coordinated against any antigen in the pathogen.

Disadvantages:

- Potential incorporation of DNA into have genome prompting insertional mutagenesis.
- Induction of immune system reactions: hostile to DNA antibodies might be created against presented DNA.
- Induction of immunologic resistance: The outflow of the antigen in the host may prompt particular nonresponsiveness to that antigen.

G. Anti-Idiotypic Vaccines: An antigen restricting site in an immunizer (paratope) is an impression of the three-dimensional structure of part of the antigen (epitope). This one of a kind amino corrosive structure in the neutralizer is known as the idiotype, which can be considered as a reflection of the epitope in the antigen. Antibodies can be raised against the idiotype by infusing the neutralizer into another creature. This against idiotype immune response mirrors some portion of the three dimensional structure of the antigen. This can be utilized as an antibody. At the point when the counter idiotype immune response is infused into a vaccinee, antibodies (antianti-idiotype antiobodies) are framed that perceive a structure like piece of the infection and may conceivably kill the infection.

Advantage:

- Antibodies against conceivably critical antigen can be delivered.

Disadvantage:

- Only humoral insusceptibility is delivered. There is no cell insusceptibility and poor memory. ID and planning of idiotypes is relentless, concentrated and troublesome.

H. Conjugate Vaccines: Conjugate immunizations are essentially created against capsulated microorganisms. While the cleansed capsular antigen can go about as subunit immunization, they invigorate just humoral invulnerability. Certain microscopic organisms have polysaccharide external coats that are ineffectively immunogenic. By connecting these external coats to proteins (e.g., poisons), the safe framework can be directed to perceive the polysaccharide as though it were a protein antigen. Polysaccharide antigens are T free, they create fleeting insusceptibility. Invulnerability to these creatures requires opsonizing antibodies. Newborn children can't mount great T-autonomous reactions to polysaccharide antigens. By covalently

connecting the polysaccharides to protein transporters, they are changed over into T-subordinate antigens and defensive resistance is incited.

Examples: Haemophilus influenzae HiB polysaccharide is complexed with diphtheria toxoid. Tetramune antibody, which consolidates the lockjaw and diphtheria toxoids, entire cell pertussis immunization, and H. influenzae sort bconjugate immunization.

I. **Toxoid vaccines:** A toxoid is a bacterial poison (normally an exotoxin) whose danger has been inactivated or smothered either by synthetic (formalin) or warmth treatment, while different properties, ordinarily immunogenicity, are kept up. Accordingly, when utilized amid immunization, an invulnerable reaction is mounted and immunological memory is framed against the atomic markers of the toxoid without bringing about poison instigated ailment Toxoid antibodies are produced using inactivated lethal aggravates that reason sickness as opposed to the smaller scale life form. Toxoids are utilized as immunizations since they instigate a resistant reaction to the first poison or increment the reaction to another antigen since the toxoid markers and poison markers are protected.

Examples:the lockjaw toxoid got from the tetanospasmin created by Clostridium tetani and the last caused lockjaw is immunized against by the DTaP immunization. Botulin is created by Clostridium botulinum.

J. **Experimental Vaccines:** various imaginative antibodies are likewise being developed and being used which are recorded beneath

Dendritic cell immunizations join dendritic cells with antigens keeping in mind the end goal to show the antigens to the body's white platelets, accordingly invigorating an insusceptible response. These immunizations have demonstrated some positive preparatory outcomes for treating cerebrum tumors and are additionally tried in dangerous melanoma.

Recombinant Vector ; by joining the physiology of one smaller scale living being and the DNA of the other, resistance can be made against ailments that have complex disease forms.

Immune system microorganism receptor peptide immunizations are a work in progress for a few infections utilizing models of Valley Fever, stomatitis, and atopic dermatitis. These peptides have been appeared to adjust cytokine creation and enhance cell-interceded resistance.

Focusing of distinguished bacterial proteins that are associated with supplement restraint would kill the key bacterial destructiveness instrument.

K. **Heterotypic Vaccines:** Also known as heterologous or "Jennerian" immunizations, these are antibodies that are pathogens of different creatures that either don't cause infection or cause gentle malady in the living being dealt with. The great illustration is Jenner's utilization of cowpox to secure against smallpox. A present case is the utilization of BCG immunization produced using Mycobacterium bovis to ensure against human tuberculosis.

7.3 IMMUNODIAGNOSTICS/SEROLOGY

Immunodiagnostics is a symptomatic procedure that uses an antigen-counter acting agent response as their essential methods for location. It is a biochemical test that measures the nearness or grouping of a macromolecule or a little particle in an answer using a neutralizer or an antigen. Serology is the investigation of serum and other organic liquids, for the symptomatic recognizable proof of antibodies in the serum. Such antibodies are regularly framed because of a disease, against other remote proteins, or to one's own proteins i.e. immune system sickness. Serological tests are performed for indicative purposes when a contamination is suspected, by checking a person's blood classification. Serology blood tests help to determine patients to have certain insusceptible insufficiencies related with the absence of antibodies. There are a few immunodiagnosis strategies that can be utilized relying upon the antibodies being examined. These include: ELISA, agglutination, precipitation, supplement obsession, and fluorescent antibodies.

7.3.1 ELISA

The protein connected immunosorbent test (ELISA) is a test that utilizations antibodies and shading change to distinguish a substance. ELISA is a "wet-lab" sort scientific organic chemistry examine that uses a strong stage catalyst immunoassay (EIA) to distinguish the nearness of a substance, as a rule an antigen, in a fluid example or wet specimen. ELISA is a plate based examines procedure which is utilized for recognizing and evaluating substances, for example, peptides, proteins, antibodies and hormones. A chemical conjugated with a counter acting agent responds with dreary substrate to create a hued item. Such substrate is called chromogenic substrate. Various catalysts have been utilized for ELISA, for example,

basic phosphatase, horse radish peroxidase and beta galactosidase. Particular substrate get hydrolysed by compounds to give hued finished result.

As an expository organic chemistry measure, the serum is brooded in a well, and each well contains an alternate serum. Antibodies or antigens introduce in serum are caught by comparing antigen or neutralizer covered on to the strong surface. After some time, the plate is washed to evacuate serum and unbound antibodies or antigens with a progression of wash cradle. To distinguish the bound antibodies or antigens, an auxiliary immune response that is joined to a catalyst, for example, peroxidase or soluble phosphatase is added to each well. After a hatching period, the unbound optional antibodies are washed off. At the point when an appropriate substrate is included, the catalyst responds with it to deliver shading. This shading delivered is quantifiable as a capacity or amount of antigens or antibodies show in the given specimen. The power of shading/optical thickness is measured at 450nm. The force of the shading gives a sign of the measure of antigen or counter acting agent. Traditionally, as different types of immunoassays, the specificity of antigen-neutralizer sort response is utilized in light of the fact that it is anything but difficult to raise a counter acting agent particularly against an antigen.

Types:

There are 3 sorts of ELISA on the premise of restricting structure between the Antibody and Antigen ELISA i.e. Coordinate, Sandwich and Competitive.

(i) Indirect ELISA: Antibody can be distinguished or quantitatively controlled by roundabout ELISA. In this procedure, antigen is covered on the microtiter well. Serum or some other example containing essential counter acting agent is added to the microtiter well and permitted to respond with the covered antigen. Any free essential immune response is washed away and the bound counter acting agent to the antigen is identified by including a protein conjugated optional neutralizer that ties to the essential immunizer. Unbound auxiliary immune response is then washed away and a particular substrate for the catalyst is included. Protein hydrolyzes the substrate to shape shaded items. The measure of shaded finished result is measured by spectrophotometric plate perusers that can gauge the absorbance of the considerable number of wells.

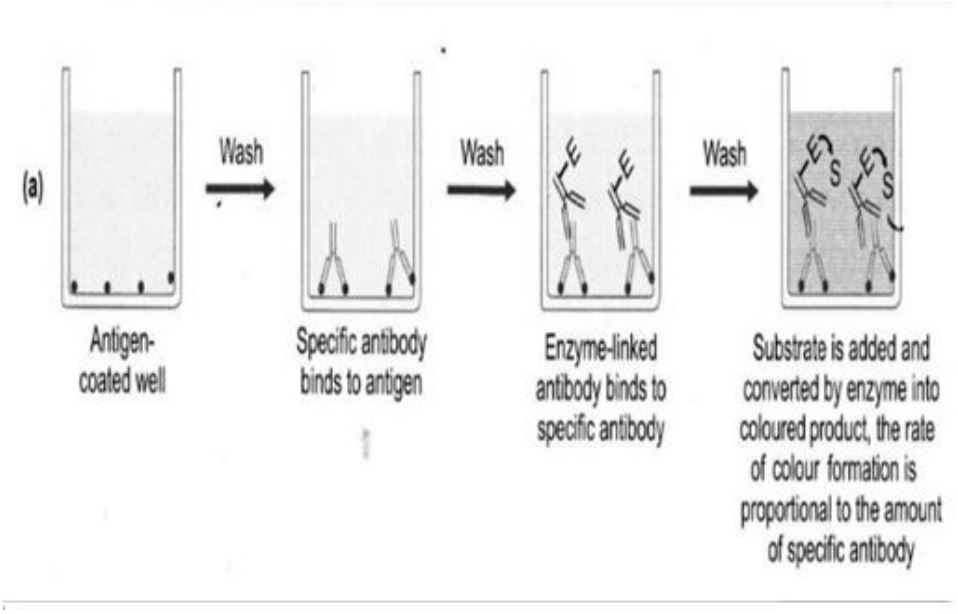


Fig.7.2 Indirect ELISA

(i) Sandwich ELISA: Antigen can be recognized by sandwich ELISA. In this system, counter acting agent is covered on the microtiter well. A specimen containing antigen is added to the well and permitted to respond with the counter acting agent connected to the well, shaping antigen-neutralizer complex. After the well is washed, a moment protein connected counter acting agent particular for an alternate epitope on the antigen is added and permitted to respond with the bound antigen. At that point after unbound auxiliary neutralizer is evacuated by washing. At long last substrate is added to the plate which is hydrolyzed by compound to frame hued items.

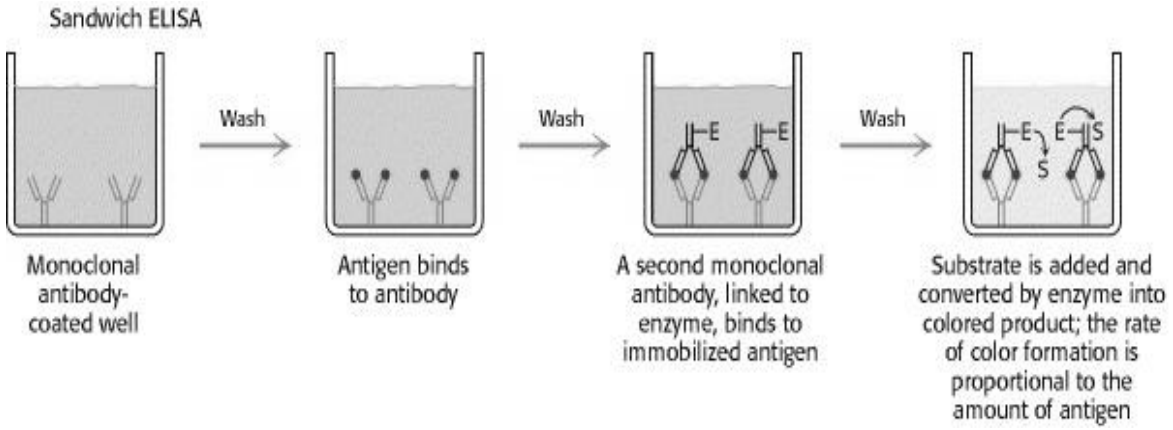


Fig.7.3 Sandwich ELISA

(ii) Competitive ELISA: This test is utilized to gauge the grouping of an antigen in a specimen. In this test, counter acting agent is first hatched in arrangement with an example containing antigen. The antigen-counter acting agent blend is then added to the microtitre well which is covered with antigen. The more the antigen exhibit in the example, the less free counter acting agent will be accessible to tie to the antigen-covered well. After the well is washed, chemical conjugated optional neutralizer particular for isotype of the essential immune response is added to decide the measure of essential immunizer bound to the well. The higher the centralization of antigen in the specimen, the lower the absorbance.

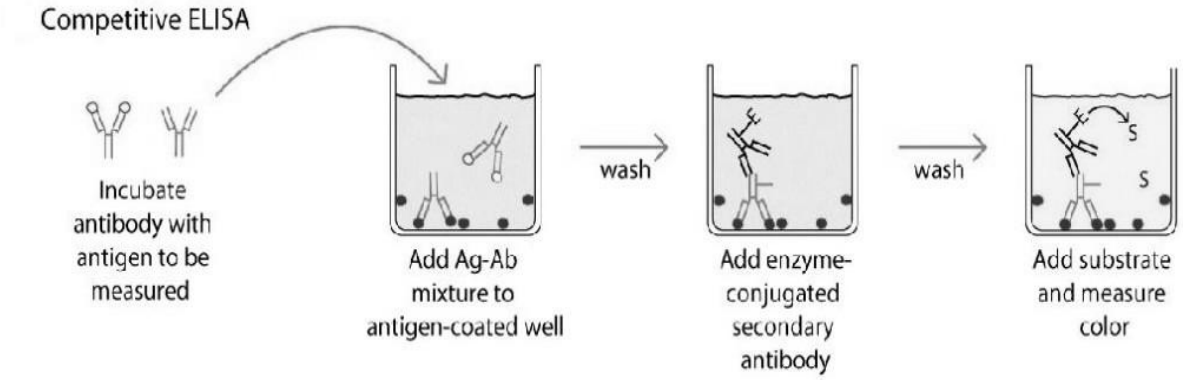


Fig 7.4Competitive ELISA

The ELISA can be performed to assess either the nearness of antigen or the nearness of counter acting agent in a specimen, it is a valuable instrument for deciding serum immunizer fixations i.e. HIV test and West Nile infection. It is additionally utilized as a part of the nourishment business in recognizing potential sustenance allergens, for example, drain,

peanuts, walnuts, almonds, and eggs and as serological blood test for coeliac illness. ELISA is likewise utilized as a part of toxicology as a fast possible screen for specific classes of medications. Alternate employments of ELISA incorporate discovery of Mycobacterium antibodies in tuberculosis, recognition of rotavirus in defecation, identification of hepatitis B markers in serum, location of enterotoxin of E. coli in defecation, location of HIV antibodies in blood tests.

7.3.2 AGGLUTINATION

Agglutination is the bunching of particles. Agglutination is the obvious articulation of the total of antigens and antibodies. Expansive antigens, conveying numerous epitopes, effortlessly sedimented particles, for example, creature cells, erythrocytes, or microscopic organisms when blended with particular antibodies, at fitting temperature and ionic quality arrangement result in cross-connecting the particles, framing a grid like structure seen as clusters with stripped eye. This response which is delicate and particular is named agglutination. Agglutination is a serological response; most basic case of agglutination is the trying for blood gathering. The immune response ties numerous antigen particles and goes along with them, making a vast cross section like complex. Agglutination is amassing together in suspension of cells bearing the antigen (epitopes)/antigen bearing microorganisms, or particles within the sight of particular antibodies called "agglutinins". A counter acting agent is a "Y" formed atom. The two arms of "Y" are the Fab part and has the consolidating site and is made of the hyper-variable locales of the substantial and light chains. The antigenic determinant settles in a separated framed by the joining site of the immune response. So the antigenic determinant fits onto the parted shaped by the "Fab". In the event that the fit is suitable then agglutination will happen. This idea is valid for all antigen (Ag) counter acting agent (Ab) responses. The procedure of agglutination includes two stages. Initial step is sharpening and second is cross section arrangement.

Sensitization: It is connection of particular immunizer to comparing antigen; pH, temperature and time of hatching impact the response. IgM antibodies respond best at 4 to 22 degrees C and IgG antibodies respond best at 37 degrees C. Time of hatching can run from 15 to a hour.

Lattice formation: Lattice is much the same as a "Jaal". It is shaped by cross connecting between sharpened particles. It requires more investment than sharpening and we might have

the capacity to see the outcome with stripped eyes. IgM best at this kind of response due to substantial size however IgG antibodies may require upgrade.

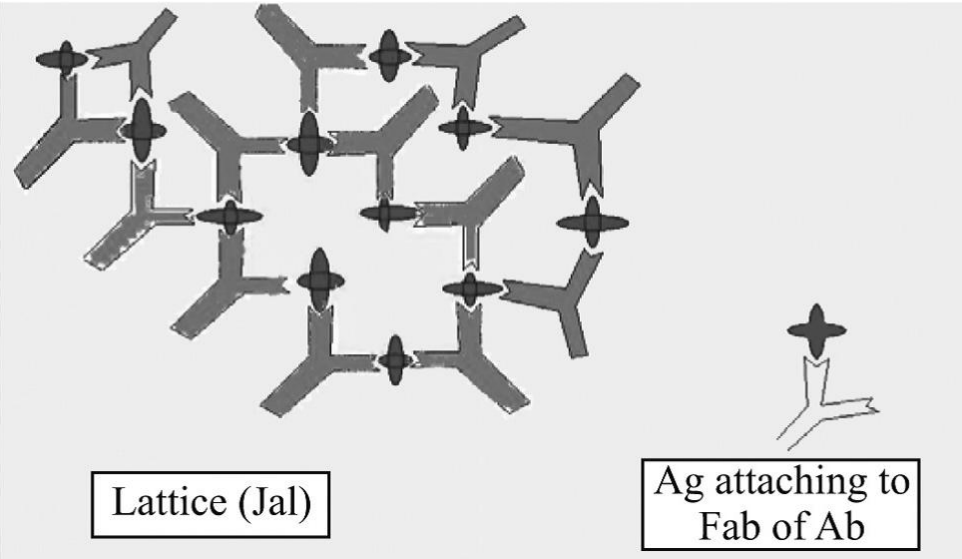


Fig.7.5 agglutination

7.3.3 IMMUNOPRECIPITATION

Immunoprecipitation (IP) is the method of accelerating a protein antigen out of arrangement utilizing a counter acting agent that particularly ties to that specific protein. This procedure can be utilized to disengage and think a specific protein from an example containing a large number of various proteins. Immunoprecipitation requires that the immunizer be coupled to a strong substrate. The two general strategies for immunoprecipitation are the immediate catch technique and the backhanded catch strategy.

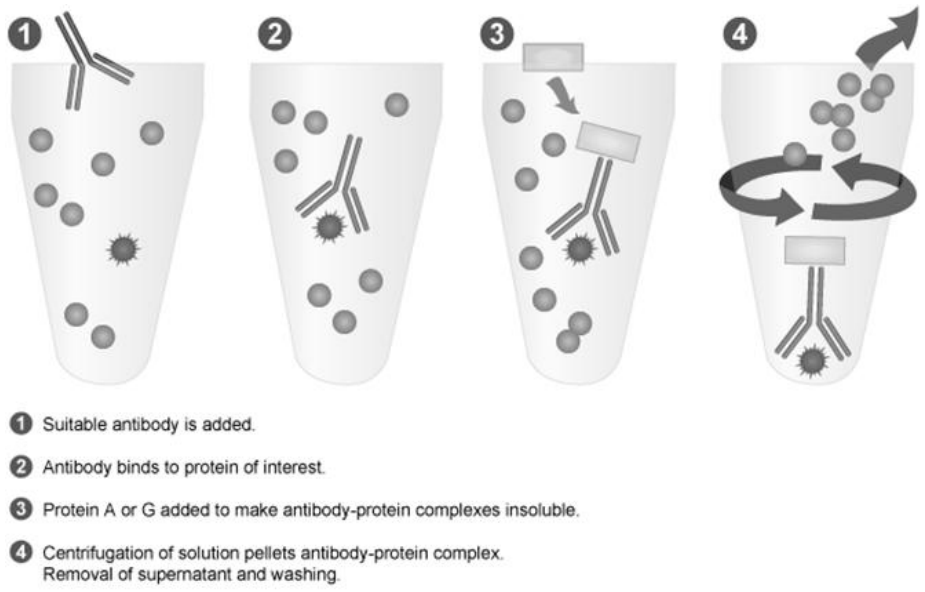


Fig 7.6 Illustration of Immunoprecipitation Process

- **Direct:** Antibodies that are particular for a specific protein or gathering of proteins are immobilized on a strong stage substrate, for example, superparamagnetic microbeads or on tiny agarose (non-attractive) dots. The globules with bound antibodies are then added to the protein blend, and the proteins that are focused by the antibodies are caught onto the dabs through the antibodies; i.e. they move toward becoming immunoprecipitated
- **Indirect:** Antibodies that are particular for a specific protein, or a gathering of proteins, are added specifically to the blend of protein. The antibodies don't connected to a strong stage bolster yet. The antibodies are allowed to drift around the protein blend and tie their objectives. Over the long haul, the dots covered in protein A/G are added to the blend of counter acting agent and protein. Now, the antibodies, which are currently bound to their objectives, will adhere to the dots.
- **Types of immunoprecipitation**

a) **Individual protein immunoprecipitation (IP):** IP includes a counter acting agent that is particular for a known protein to detach that specific protein out of an answer containing various proteins. These arrangements are regularly as a rough lysate of a plant or creature tissue.

b) **Protein complex immunoprecipitation (Co-IP):** Immunoprecipitation of in place protein buildings is known as co-immunoprecipitation (Co-IP). Co-IP works by choosing a counter acting agent that objectives a known protein that is an individual

from a bigger complex of proteins. By focusing on this known part with an immune response which makes it conceivable to haul the whole protein complex out of arrangement and along these lines distinguish obscure individuals from the complex. Co-IP is an intense method; this works when the proteins associated with the intricate tie to each other firmly, making it conceivable to pull various individuals from the complex out of arrangement by hooking onto one part with an immunizer. This idea of hauling protein edifices out of arrangement is likewise alluded as a "draw down".

c) **Chromatin immunoprecipitation (ChIP):** (ChIP) is utilized to decide the area of DNA restricting locales on the genome for a specific protein of intrigue. This method gives a photo of the protein– DNA cooperations that happen inside the core of living cells or tissues. In these test DNA-restricting proteins (counting translation elements and histones) in living cells are cross-connected to the DNA that they are authoritative. By utilizing a counter acting agent that is particular to a putative DNA restricting protein, immunoprecipitate; the protein– DNA complex out of cell lysates. The crosslinking is proficient by applying formaldehyde to the cells (or tissue), or DTBP. Following crosslinking, the cells are lysed and the DNA is broken into pieces 0.2– 1.0 kb long by sonication. Now the immunoprecipitation is performed bringing about the cleansing of protein– DNA buildings. The cleansed protein– DNA edifices are then warmed to turn around the formaldehyde cross-connecting of the protein and DNA buildings, enabling the DNA to be isolated from the proteins. To personality and amount the DNA parts disconnected is dictated by PCR.

d) **RNP Immunoprecipitation (RIP):** It is like chromatin immunoprecipitation (ChIP), but instead than focusing on DNA restricting proteins as in ChIP, a RNP immunoprecipitation targets ribonucleoproteins (RNPs). Live cells are first lysed and after that the objective protein and related RNA are immunoprecipitated utilizing a counter acting agent focusing on the protein of intrigue. The sanitized RNA-protein buildings are isolated by playing out a RNA extraction and the recognizable proof of the RNA is dictated by cDNA sequencing or RT-PCR.

Labeled proteins: The constraints of all the previously mentioned IP tests is their reliance upon the accessibility of antibodies that uncommonly perceive the objective protein with practically zero cross reactivity with other cell targets. Because of this constraint, numerous proteins can't be IP due to the absence of an accessible counter acting agent. To handle this issue, this test utilizes labeling of proteins with an epitope

to which a high proclivity counter acting agent is accessible and ectopically communicated in the phone of intrigue. These labels can be either short peptide groupings or fluorescent proteins like Flag, c-Myc, hemagglutinin (HA) and Green fluorescent protein (GFP). The upside of labeled proteins is that same tag can be utilized on numerous occasions on a wide range of proteins and it can be utilized for a similar immunizer each time.

7.3.4 COMPLEMENT-FIXATION

The supplement obsession test is an immunological restorative test that is utilized to distinguish the nearness of either particular immunizer or particular antigen in a patient's serum, in light of supplement obsession. The supplement framework is an arrangement of serum proteins that respond with antigen-counter acting agent buildings. The response happens on a cell surface, it brings about the development of trans-film pores and consequently annihilation of the phone. Most importantly serum is isolated from the patient. Patients normally have distinctive levels of supplement proteins in their serum. To refute any impacts this may have on the test, the supplement proteins in the patient's serum must be devastated and supplanted by a known measure of institutionalized supplement proteins. The serum is warmed such that the greater part of the supplement proteins yet none of the antibodies inside it are obliterated. A known measure of standard supplement proteins is added to the serum. The antigen of intrigue is added to the serum. Sheep red platelets (sRBCs) which have been pre-bound to hostile to sRBC antibodies are added to the serum. The test is viewed as negative if the arrangement turns pink now and positive generally. On the off chance that the patient's serum contains antibodies against the antigen of intrigue, they will tie to the antigen to frame antigen-counter acting agent edifices. The supplement proteins will respond with these buildings and be exhausted. Along these lines when the sRBC-neutralizer buildings are included, there will be no supplement left in the serum. Be that as it may, if no antibodies against the antigen of intrigue are available, the supplement won't be drained and it will respond with the sRBC-immune response buildings, lysing the sRBCs and spilling their substance into the arrangement, along these lines turning the arrangement pink.

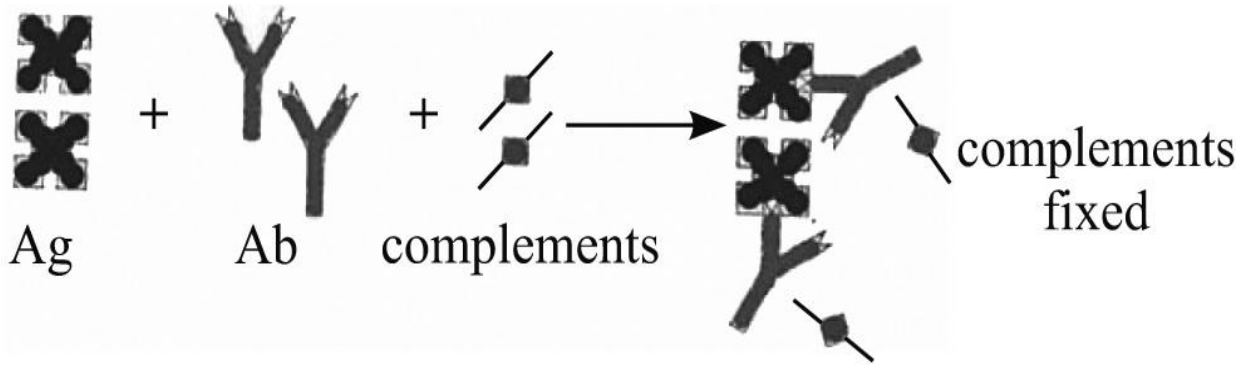


Fig.7.7 Complement fixation

7.3.5FLUORESCENT ANTIBODIES/IMMUNOFLUORESENCE

A fluorescent antibody (FA) otherwise called "immunofluorescence". ImmunoFluorence is characterized as a method utilized for identifying an antigen or counter acting agent in an example by coupling its particularly intuitive immunizer or antigen to a fluorescent color/compound, blending with the specimen, and after that watching the response under a bright light fluorescence magnifying lens. Its name gets from the way that it tests the nearness of an antigen with the labeled neutralizer, not at all like western smearing, which utilizes a circuitous technique for recognition, where the essential counter acting agent ties the objective antigen, with an optional immune response coordinated against the essential, and a label connected to the auxiliary immunizer. Fluorescent-neutralizer systems utilize antibodies named with fluorescent colors. Fluorescence is a kind of glow. The fluorescent colors/fluorochromes (having luminescent properties) ingest light of one wavelength and transmit light of an alternate wavelength to a great degree quickly. Consumed light has a higher vitality than the transmitted fluorescence light, so the wavelength of the produced light is longer than that of the excitation light. Blue light is the excitation range and green light is the emanation range. A molecule has electrons each of which has foreordained level of vitality. An electron can retain vitality from a photon of light and end up plainly energized. The vitality level is higher in the energized organize, yet this stage is temperamental. The energized electron radiates fluorescence; the vitality of electron at this stage is lower than when in energized organize. This creates the amplified fluorescent picture of the protest which can be imagined with fluorescence magnifying instrument.

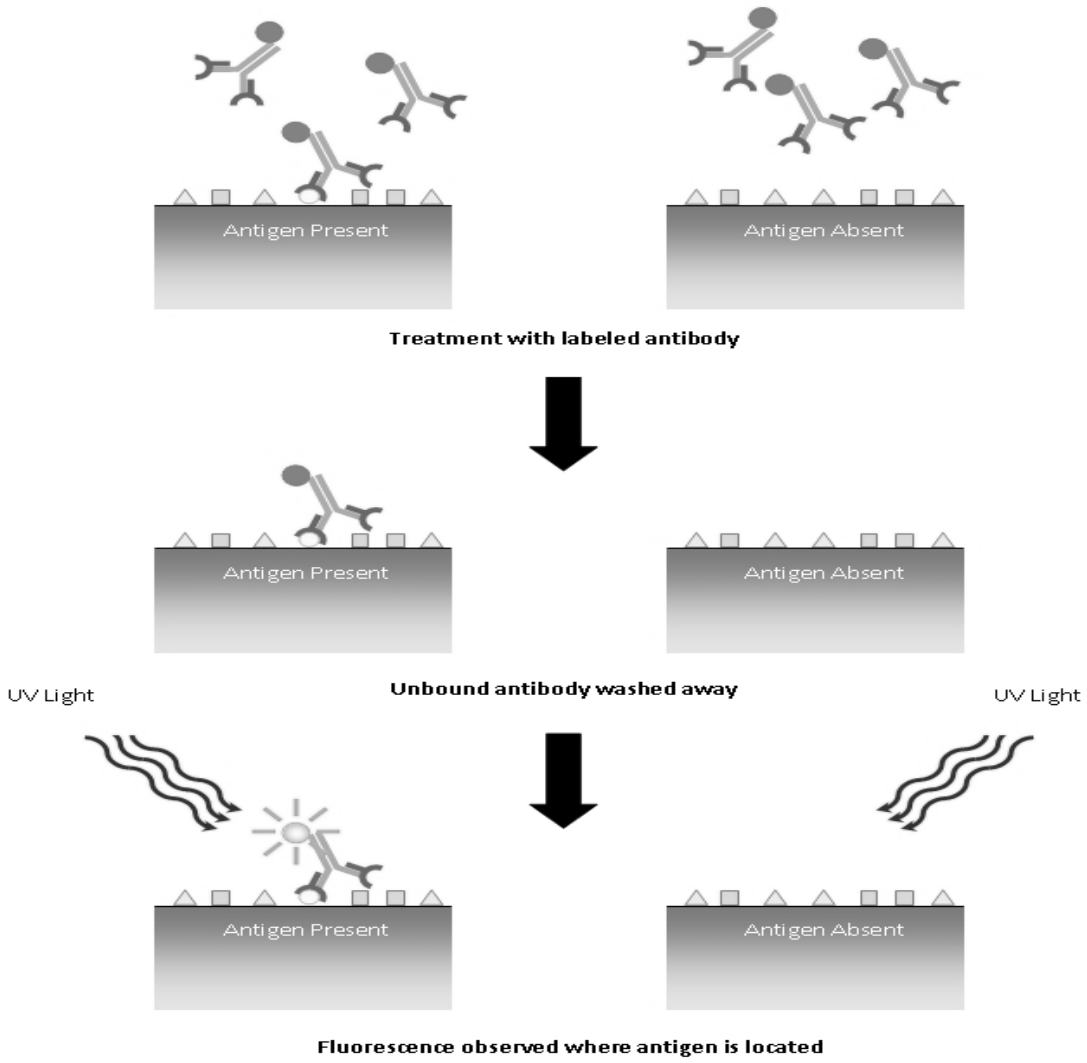


Fig 7.8

It is of two types direct & indirect:

- **Direct Fluorescent-Antibody Tests:** Direct fluorescent-antibody tests utilized to distinguish particular microorganisms (antigens). Antibodies coordinated against antigens on the surface of a particular microorganism are marked with fluorescent color. Fluorescent antibodies are hatched with the example and antigen-particular restricting permitted to happen. Overabundance and non-particularly connected antibodies are washed from the specimen. The example is seen with a fluorescence watcher, regardless of whether a fluorescence magnifying lens or plate peruser or even fluorescence-enacted stream cytometer.

- **Indirect Fluorescent-Antibody Tests:** Indirect fluorescent-counter acting agent tests are utilized to exhibit the nearness of antibodies against a particular antigen in serum. Antigen or the microorganism itself is hatched with the patient's serum. Overabundance serum is washed away, leaving just antibodies particular for the antigen (or antigenic segment of the microorganism) exhibit in the patient's serum bound. The example is then brooded with antibodies named with fluorescent color that are particular for human antibodies (fluorescent aniti-human neutralizer antibodies), (infuse human immunoglobulins into another species and it will create against human immunoglobulin antibodies). The example is seen with a fluorescence watcher, precisely like the immediate counter acting agent tests.

7.4 IMMUNOTHERAPY

Immunotherapy is the "treatment of ailment by initiating, upgrading, or stifling a resistant reaction. Immunotherapy is treatment that uses certain parts of a man's invulnerable framework to battle sicknesses. Immunotherapy is treatment that is intended to saddle the capacity of the body's resistant framework to battle contamination or illness. Immunotherapy may deliver an invulnerable reaction to ailment or improve the safe framework's protection from dynamic maladies, for example, malignancy. Some of the time alluded to as natural treatment, immunotherapy regularly utilizes substances alluded to as organic reaction modifiers (BRMs). The body typically just delivers little measures of these BRMS in light of contamination or malady, however in the research center, a lot of these BRMs can be created with a specific end goal to give a treatment. Contingent upon the sort of treatment, different reactions can emerge because of utilizing immunotherapy. Reactions incorporate influenza like side effects, muscle hurts, fever, craving misfortune, shortcoming, loose bowels, queasiness and retching. A rash may create and a few people wound or drain effortlessly. These symptoms are for the most part here and now.

Eg. of immunotherapies incorporate monoclonal antibodies, interferon, interleukin-2 (IL-2), and settlement invigorating components CSF, GM-CSF and G.

Immunotherapies intended to inspire or open up an insusceptible reaction are delegated enactment immunotherapies, while immunotherapies that lessen or smother are named concealment immunotherapies.

7.4.1 ACTIVATION IMMUNOTHERAPIES

An initiation immunotherapy is one that either actuates or enhances an invulnerable reaction. These are of following strategies:

7.4.2 FOR CANCER

Cancer immunotherapy endeavors to invigorate the resistant framework to devastate tumors. Dendritic cells or DCs are powerful antigen-creating cells that can sharpen T cells to both new and review antigens. The objectives of DC based immunotherapy in tumor are to prime particular antitumor resistance by creating effector lymphocytes (e.g. CD4+ T cells and CD8+ T cells) that objective, assault and obliterate tumors.

7.4.3 DENDRITIC CELL BASED IMMUNOTHERAPY

Dendritic cells are fortified to actuate a cytotoxic reaction towards an antigen. Dendritic cells, a kind of antigen showing cell, are gathered from the individual requiring the immunotherapy. These cells are then either beat with an antigen or tumor lysate[17] or transfected with a viral vector, making them show the antigen. Upon transfusion into the individual, these enacted cells introduce the antigen to the effector lymphocytes (CD4+ assistant T cells, cytotoxic CD8+ T cells and B cells). This starts a cytotoxic reaction against tumor cells communicating the antigen (against which the versatile reaction has now been prepared).

7.4.4 T CELL BASED RECEPTIVE IMMUNOTHERAPY

Another immunotherapy strategy that outfits a patient's resistant framework keeping in mind the end goal to battle infection is assenting cell treatment (ACT). This includes building a patient's T cells with the goal that they can perceive and assault tumor cells. Immune system microorganisms are gathered from the patient's blood and after that hereditarily built to deliver cell surface receptors alluded to as illusory antigen receptors (CARs). Autos empower the T cells to perceive particular antigens exhibit on the surface of malignant cells. The T cells then increase in the body and by utilizing their hereditarily designed receptor; they assault cells that present the tumor particular antigen on their surface.

7.4.5 IMMUNE UPGRADE TREATMENT

- It utilizes a man's own particular fringe blood-determined common executioner cells, cytotoxic T lymphocytes and other applicable resistant cells are extended in vitro and afterward reinfused.
- Genetically designed T cells: These phones are made by reaping T cells and afterward contaminating the T cells with a retrovirus that contains a duplicate of a T cell receptor (TCR) quality that is specific to perceive tumor antigens. The infection coordinates the receptor into the T cells' genome. The phones are extended non-particularly as well as invigorated. The cells are then reinfused and deliver a safe reaction against the tumor cells.

7.4.6 SUPPRESSION IMMUNOTHERAPIES

Therapies that lessen an invulnerable reaction to treat hypersensitivity or immune system ailments are alluded to as concealment immunotherapies. Safe concealment hoses an irregular invulnerable reaction in immune system ailments or lessens an ordinary insusceptible reaction to avoid dismissal of transplanted organs or cells. These are of following techniques:

7.4.7 IMMUNOTHERAPY FOR SENSITIVITY

Immunotherapy is utilized to treat hypersensitivities. Hypersensitivity medications treat unfavorably susceptible side effects, immunotherapy decrease affectability to allergens, diminishing its seriousness. It delivers long haul benefits. Immunotherapy is somewhat powerful in a few people and ineffectual in others, yet it offers sensitivity sufferers an opportunity to lessen or stop their side effects. The treatment is shown for individuals who are to a great degree unfavorably susceptible or who can't evade particular allergens. Immunotherapy is for the most part not demonstrated for sustenance or therapeutic sensitivities. This treatment is especially helpful for individuals with hypersensitive rhinitis or asthma. The main measurements contain small measures of the allergen or antigen. Measurements increment after some time, as the individual moves toward becoming

desensitized. Allergen immunotherapy works by smothering this second stage reaction and by lessening the primary stage reaction by forestalling allergen-driven Th2 reactions, which incorporates a fall in the levels of interleukin. Allergen immunotherapy brings about a long haul diminish in serum allergen-particular IgE levels and furthermore that the early stage reaction is essentially decreased.

7.4.8 IMMUNOTHERAPY FOR TRANSPLANT PATIENTS

All patients who get an organ or tissue transplant (allograft) need to take immunosuppressive treatments to keep the body perceiving the organ as remote and propelling a resistant reaction to assault it. Allogeneic transplant won't be fruitful unless the patient's invulnerable framework is downregulated and this downregulation should likewise be kept up in the long haul. Immunosuppressive treatment downregulate the insusceptible framework such that with or without different reactions from those to the allograft stay in place.

7.4.9 IMMUNOTHERAPY FOR AUTOIMMUNE SICKNESS

Disorders of the invulnerable framework make the insusceptible framework turn out to be either overactive or underactive. In situations where the resistant framework is overactive, it produces antibodies which assault and harm the body's own tissues as opposed to battling contamination and this is named as immune system sickness. Immune system issue that can be treated with immunosuppressant treatments incorporate rheumatoid joint pain and fiery inside ailment.

7.4.10 RHEUMATOID JOINT PAIN

In rheumatoid joint pain, antibodies are delivered by the safe framework that assaults the linings in joints. Individuals with direct to extreme rheumatoid joint pain in this way as a rule need to take pharmaceutical. In cases, the disease modifying antirheumatic drug (DMARD) methotrexate and if that neglects to work, an option DMARD, for example, hydroxychloroquine or sulfasalazine might be endorsed. In situations where DMARDs neglect to mitigate manifestations, a suppressive immunotherapy is suggested. These built proteins square specific parts of the resistant reaction that prompts aggravation and moderate or end the movement of rheumatoid joint inflammation.

7.5 SUMMARY

Vaccine is arrangement of pathogenic specialists or its constituent part, which can be managed with the target of animating defensive resistance. Immunizations that are usually utilized incorporate weakened antibody, inactivated antibody, toxoid immunization and polysaccharide immunization. Constricted immunizations utilize debilitated pathogen to invigorate insusceptible arrangement of the antibody. Since, pathogens are constricted or debilitated they once in a while cause harm. Inactivated antibody utilize slaughtered pathogen while toxoid immunization utilize synthetically adjusted poison subsidiary that has lost danger yet at the same time holds immunogenicity. Capsular polysaccharides of microscopic organisms have likewise been utilized as immunization with fantastic outcomes. In making recombinant antigen immunization, quality coding for antigen is brought into have cell (yeast, microorganisms) utilizing recombinant DNA innovation. The protein is then communicated and gathered and utilized as immunization. Live vector immunization (bacterial or viral) fill in as wellspring of antigen inside the antibody. Antigen qualities are presented in weakened microorganisms or safe infection and are tainted into the host framework. Antigens shaped and discharged by these vectors empower both B-cell and T-cell intervened resistance. DNA antibody includes coordinate presentation of antigen quality into muscle cell or antigen displaying cell. Target quality as quality gold molecule adduct is besieged on the cell by quality weapon. Once inside, quality is communicated and antigen showed on have cell MHC that empowers invulnerable framework. A perfect immunization should (a) furnish long lasting resistance with a solitary measurement, (b)be non-intrusive (c) invigorate both humoral and cell-interceded insusceptibility (d) be shabby and simple to store and transportAgglutination is utilized for analysis of ailments in lab either utilizes the particulate or solvent antigens. The pointer framework is shaped by sheep red platelets covered with rabbit immunizer to sheep red cells (amboceptors). The sheep red platelets will lyse within the sight of supplement. There exits alteration of supplement obsession test- Indirect supplement obsession test, Congulatinating supplement retention test, Immune adherence, Immobilization test, Cytolytic tests. Fluorescence colors are conjugated with antibodies and these marked antibodies are utilized as tests to recognize and find the antigen particular to this neutralizer. Coordinate immunofluorescence is utilized less much of the time as the counter acting agent against the atom of intrigue is synthetically conjugated to a fluorescent color, so for each antigen to be distinguished, the particular neutralizer should be conjugated with FITC. In backhanded fluorescence the neutralizer particular for the atom of

intrigue (called the essential counter acting agent) is unlabeled, and a moment hostile to immunoglobulin immunizer coordinated toward the consistent bit of the main counter acting agent (called the optional counter acting agent) is labeled with the fluorescent color. Aberrant fluorescence is utilized all the more normally as the labeled auxiliary neutralizer can be utilized to distinguish a wide range of antigens. Nonetheless, the essential counter acting agent should be particular for the antigen to be distinguished. Applications incorporate; Resolution of points of interest to the atomic level, Study a cell populace for reasonability (some fluorophores infiltrate live cells and not the dead cells as of now clarified under microscopy), Detect particular cells of enthusiasm for an example/material utilizing FISH methods.

7.6 GLOSSARY

Ablative treatment: the use of heat, extreme cold, lasers, or a chemical to destroy cancerous tissue.

Active immunity: The production of antibodies against a specific disease by the immune system

Antibiotic: A substance that fights bacteria.

Antitoxin: Antibodies capable of destroying toxins generated by microorganisms including viruses and bacteria

Antiviral: any medicine capable of destroying or weakening a virus

Array: Diagnostic modality involving conduction of multiple unique tests for different biomarkers on the same testing medium

Asymptomatic infection: The presence of an infection without symptoms.

Attenuated vaccine: A vaccine in which live virus is weakened through chemical or physical processes in order to produce an immune response without causing the severe effects of the disease

Biomarkers: are proteins or genes that provide a more detailed understanding of the tumor, its prognosis, and the potential response to treatment.

Biopsy: a procedure in which a doctor removes a small sample of tissue

Booster shots: Additional doses of a vaccine needed periodically to "boost" the immune system

Chemotherapy: often called "chemo," is a treatment with drugs that kill cancer cells

Cholangiopancreatography: is the use of endoscopy combined with fluoroscopy to diagnose cancerous tumors in the pancreas

Combination vaccine: Two or more vaccines administered in a single dose in order to reduce the number of shots given

Conjugate vaccine: The joining together of two compounds (usually a protein and polysaccharide) to increase a vaccine's effectiveness.

Deltoid: A muscle in the upper arm where shots are usually given

Dx: Dx is the abbreviation for diagnostics

Efficacy rate: A measure used to describe how good a vaccine is at preventing disease

Endoscopic ultrasound: uses a thin, flexible tube called an endoscope that has a built-in miniature ultrasound probe to explore the digestive tract and create visual images with sound waves.

Fluorescence in situ hybridization: or FISH, is a screening test that can be performed on breast cancer tissue removed during a biopsy

Hematology: The scientific study of blood and blood-forming tissues.

Hormone-lowering therapy: also called androgen deprivation therapy or androgen suppression therapy, to reduce levels of male hormones (androgens) in the body.

Immune modulators: are drugs used to “step on the gas” of the immune response, allowing the body to respond more aggressively to cancer

Immunization: The process by which a person or animal becomes protected against a disease

Immunoassay: Tests used to detect or quantify a specific substance, the analyte, in a blood or body fluid sample, using an immunological reaction.

Immunochemistry: The study of the chemical properties of antigens and antibodies, complement, and T cell receptors

Immunodiagnostics: The use of specific antibodies to measure a substance

Immunosupression: When the immune system is unable to protect the body from disease

Immunotherapy: one of the most promising and scientifically advanced cancer treatments

Interferons: IFN, type of cytokine that boosts the ability of certain immune cells to attack invaders like viruses or cancer cells.

Interleukins: IL, are cytokines that help immune cells grow and divide more quickly

Investigational vaccine: A vaccine that has been approved by the Food and Drug Administration (FDA) for use in clinical trials on humans

Melanoma: is a type of skin cancer that begins in skin cells called melanocytes

Polysaccharide vaccines: Vaccines that are composed of long chains of sugar molecules that resemble the surface of certain types of bacteria

Quarantine: The isolation of a person or animal who has a disease (or is suspected of having a disease) in order to prevent further spread of the disease

Thimerosal: Thimerosal is a mercury-containing preservative used in some vaccines and other products

Titer: The detection of antibodies in blood through a laboratory test

Tumor: is an abnormal lump or mass of tissue

Vaccination: Injection of a killed or weakened infectious organism in order to prevent the disease

Vaccine: A product that produces immunity therefore protecting the body from the disease

Vaccinia: A virus related to the smallpox and cowpox viruses, which is used in smallpox vaccine

7.7 SELF ASSESSMENT QUESTION

Multiple Choice Questions

1.DNA vaccines can be effective if they

A. can be engineered to contain DNA motifs that have an adjuvant effect.

B. encode expression of antigen.

C. encode expression of appropriate cytokines.

D. all of the above.

2. Polysaccharides are rarely effective vaccines by themselves because they …

A. have repeating B cell epitopes. B. lack classical T cell epitopes.

C. only induce CTL responses. D. are usually the same in people and bacteria.

3. Vaccines may fail to induce a protective response because they induce …

A. humoral immunity when cell mediated immunity is needed.

B. IgM but not IgG or IgA.

C. production of IL-4 when IFNγ is needed

D. all of the above.

4. Most chemotherapeutic drugs kill cells by damaging

A. DNA B. Protein

C. Nearby blood vessels D. All of these

5. Sigmoidoscopy is a test for

A. Breast cancer B. Colon cancer

C. Cervical cancer D. Brain cancer

Answers:

1. All of the above 2. Lack classical T cell epitopes

3. All of the above 4. DNA

5. Colon cancer

Fill in the blanks:

1. The process of introduction of weakened pathogen into human body is called_____.

2. The first vaccine was developed by _____.

3. _____can is accelerated by a previous graft from the same donor.

4. _____is technique used for detecting antigen/antibody by coupling them with fluorescent dye.

5. Attachment of specific antibody to antigen is_____.

Answer:

1. Vaccination 2. Louis Pasteur

3. Graft rejection 4. Immunofluroescence

5. Sensitization

Short Answer Type Questions

1. What do you understand by the term complement?

Ans. It is an immunological medical test that is used to detect the presence of either specific antibody or specific antigen in a patient's serum.

2. Name auto fluorescent structures?

Ans. Auto fluorescent structures are Mitochondria, riboflavin & collagen.

3. Define ELISA?

Ans. Enzyme-linked immunosorbent assay

4. What is Live Attenuated Vaccine?

Ans. The vaccines which are composed of live, reduced in virulence microorganisms that cause a limited infection in their hosts are termed as live attenuated vaccine.

5. Give examples of tagged proteins?

Ans. Flag, c-Myc, hemagglutinin (HA) & Green fluorescent protein (GFP).

Long Answers Type Questions

1. Explain vaccines & its types in detail with examples?

2. Define serology with the help of various techniques?

3. Describe immunoprecipitation & its types?

4. What is immunotherapy; explain in brief with suitable examples?

5. What is immunofluorescence? Describe the different types of immunofluorescence techniques?

7.8 REFERENCES

1. Janeway, Charles A., Travers, Jr., Paul, Walport, Mark and Shlomchik, Mark J. (2001).Immunobiology, 5th edition. Garland Science, New York.
2. Kind, Thomas J., Goldsby, Richard A., Osborne, Barbara Anne. and Kuby, Janis. (2007). Kuby Immunology. New York: W.H. Freeman.
3. Lydyard, P.M., Whelan, A. and Fanger, M.W., (2004). Immunology. Garland Science/BIOS Scientific Publishers Limited

Block III: Toxicology

UNIT 8: TOXIN

CONTENTS

8.1 OBJECTIVES

The objectives of the study of Toxicology will let the students to:

- Define toxicology- The study of toxic substances is known as Toxicology(effect, problem)
- What are toxins their types and sources
- Natural occurring toxins and their rout of entry into human body
- Dose- responses of toxins and their relationship

8.2 TOXINS

A term toxin (from Ancient Greek: τοξικόν toxikon) is used for a harmful substance delivered inside living cells or life forms; manufactured toxicants made by counterfeit procedures are subsequently prohibited. The term was first utilized by organic chemist **Ludwig Brieger (1849– 1919)**.

Toxins can be little atoms, peptides, or proteins that are equipped for causing infection on contact with or retention by body tissues cooperating with natural macromolecules, for example, compounds or cell receptors. Poisons fluctuate significantly in their poisonous quality, running from generally minor, (for example, a honey bee sting) to very quickly lethal, (for example, botulinum poison).

Poisons are regularly recognized from other synthetic operators by their technique for creation—the word poison does not indicate strategy for conveyance (contrast and venom and the smaller significance of toxin—all substances that can likewise make unsettling influences living beings). It essentially implies it is an organically created harm. There was a progressing expressed question between NATO (**North Atlantic Treaty Organization**) and the Warsaw Pact (**Treaty of Friendship, Co-operation, and Mutual Assistance**) about whether to call a poison a natural or synthetic operator, in which the NATO decided on natural specialist, and the Warsaw Pact, as most different nations on the planet, for concoction specialist.

As per an International Committee of the Red Cross survey of the Biological Weapons Convention, "Poisons are noxious results of living beings; dissimilar to natural operators, they are lifeless and not fit for duplicating themselves", and "Since the marking of the

Convention, there host been no question among the gatherings in regards to the meaning of organic specialists or poisons".

As indicated by Title 18 of the United States Code, "the expression "poison" implies the poisonous material or result of plants, creatures, microorganisms(including, however not constrained to, microscopic organisms, infections, parasites, rickettsiae or protozoa), or irresistible substances, or a recombinant or incorporated atom, whatever their starting point and technique for generation".

A fairly casual wording of individual poisons relates them to the anatomical area where their belongings are generally prominent:

- Hemotoxin, causes decimation of red platelets (hemolysis)
- Phototoxin, causes hazardous photosensitivity

8.3 DIFFERENT KIND OF TOXINS

There are for the most part four sorts of dangerous substances; synthetic, organic, physical and radiation:

- Chemical toxicants incorporate inorganic substances, for example, lead, mercury, hydrofluoric corrosive, and chlorine gas, and natural mixes, for example, methyl liquor, most solutions, and toxic substances from living things. While some feebly radioactive substances, for example, uranium, are additionally synthetic toxicants, all the more unequivocally radioactive materials like radium are not, their unsafe impacts (radiation harming) being caused by the ionizing radiation created by the substance as opposed to compound connections with the substance itself.
- Disease-causing microorganisms and parasites are poisonous in an expansive sense, however are for the most part called pathogens as opposed to toxicants. The natural danger of pathogens can be hard to gauge in light of the fact that the "limit dosage" might be a solitary creature. Hypothetically one infection, bacterium or worm can repeat to cause a genuine contamination. In any case, in a host with an in place safe framework the inborn danger of the living being is adjusted by the host's capacity to battle back; the compelling harmfulness is then a mix of the two sections of the relationship. Sometimes, e.g. cholera, the ailment is mostly caused by a nonliving

substance emitted by the living being, instead of the life form itself. Such nonliving organic toxicants are for the most part called poisons if delivered by a microorganism, plant, or parasite, and venoms if created by a creature.

- Physical toxicants are substances that, because of their physical nature, meddle with organic procedures. Cases incorporate coal tidy, asbestos fibbers or finely partitioned silicon dioxide, all of which can at last be lethal if breathed in. Destructive chemicals have physical lethality since they crush tissues; however they're not straightforwardly toxic unless they meddle specifically with natural movement. Water can go about as a physical toxicant if taken in to a great degree high measurement in light of the fact that the centralization of essential particles diminishes drastically if there's an excess of water in the body. Asphyxiant gasses can be viewed as physical toxicants since they act by dislodging oxygen in nature however they are idle, not synthetically lethal gasses.
- Radiation poisons are of a few sorts of a couple of sorts of particles and beams transmitted by radioactive material, high-voltage equipment, nuclear reactions, and stars. The sorts that are commonly basic to your prosperity are alpha particles, beta particles, x bars, and gamma pillars. Alpha and beta particles are nearly nothing, fast moving bits of iotas that a radioactive atom transmits when it changes into another substance. X bars and gamma bars are sorts of electromagnetic radiation. These radiation particles and shafts pass on enough imperativeness to pound out electrons from particles and molecules, (for instance, water, protein, and DNA) that they hit or go close. This system is called ionization, which is the reason this radiation is called "ionizing radiation."
- On a more extensive scale, poisons might be named either exotoxin, being discharged by a life form, or endotoxins, that are discharged for the most part when microorganisms are lysed.

Related terms are:

- Toxoid, debilitated or smothered poison
- Venom, poisons in the feeling of utilization by specific sorts of creatures

8.4 ENVIRONMENTAL TOXINS

The expression "ecological poison" can here and there unequivocally incorporate engineered contaminants, for example, modern contaminations and other misleadingly made poisonous substances. As this negates most formal meanings of the expression "poison", it is vital to affirm what the scientist implies while experiencing the term outside of microbiological settings.

Ecological poisons from evolved ways of life that might be risky to human wellbeing include:

- Paralytic shellfish harming (PSP)
- Amnesic shellfish harming (ASP)
- Diarrheal shellfish harming (DSP)
- Neurotoxin shellfish harming (NSP)

As said some time recently, there's a wide assortment of toxicants in the earth. To better comprehend them, we can place them into particular classifications that depend on the sorts of issues they cause. Cancer-causing agents are most likely the best-known toxicant in light of the fact that these are tumour causing chemicals. Tobacco smoke falls into this class as it contains more than 4,000 chemicals, a considerable lot of which cause malignancy.

a) Mutagens are change causing chemicals. At the point when living beings are presented to a mutagen, it actually changes their DNA, prompting malignancy and different issue. X-beams are outstanding mutagens. Teratogens are chemicals that reason mischief to unborn infants. The name of this toxicant originates from the Greek word teras, which implies beast.

These chemicals cause birth absconds amid improvement in the womb. Thalidomide was utilized as a part of the 1950s as a resting pill and to avoid sickness amid pregnancy, however ended up being an extremely hurtful teratogen. Indeed, even a solitary measurement is sufficiently effective to cause serious birth absconds in kids.

b) Allergens are chemicals that fortify over activity in the invulnerable framework. When you are presented to allergens, your body goes into overdrive, setting off an invulnerable reaction to attempt and dispose of the allergen. This is the reason dust and tidy reason side effects that are like being debilitated.

c) **Neurotoxins** are chemicals that assault the sensory system. These incorporate overwhelming metals, similar to lead and mercury, and additionally pesticides and synthetic weapons. Neurotoxins can prompt side effects like slurred discourse, loss of muscle control and even passing.

d) **Endocrine disrupters** are chemicals that upset the endocrine framework in life forms and regularly originate from professionally prescribed medications and chemicals in plastics. The endocrine framework is otherwise called the hormone framework, and this piece of your body is the thing that manages development, advancement, sexual development, cerebrum work and even hunger.

   Toxicants that disturb hormone working can prompt some major issues since they so intently look like genuine hormones in your body. Reptiles and creatures of land and water are particularly delicate to endocrine disrupters and introduction regularly prompts feminization of male creatures. This may appear like a great deal to recall! In any case, on the off chance that you look carefully, you'll see that the name of the toxicant depicts the impact it has on living beings, which encourages us comprehend these toxicants better.

8.5 SOURCES OF TOXIC AGENTS

We are encompassed by manufactured chemicals and experience them incalculable circumstances consistently. Plastics, family unit cleaners, solvents, cleansers, beautifying agents and aromas are on the whole toxicants. So are anti-infection agents, physician recommended drugs, steroids, sustenance added substances, additives and different things we ingest. Pesticides, herbicides and composts are likewise toxicants.

In spite of the fact that poisons originated from amny sources, they tend to travel through nature in certain ways. Toxicants may discover their way into sea-going framework as they escape by spill over from substantial ranges of land. Since the water frameworks are littler than the land that provided the contaminants, the poisons have a tendency to get packed in the water.

Sources of toxicants discharged to nature

A. **Point sources**–discrete, identifiable, particular geographic area of synthetic release, a specific stationary place of passage of chemicals into nature

(i) such as: – a particular smoke stack, a specific pipe releasing profluent into an accepting stream, a processing plant, a sewage treatment plant, a synthetic waste dump, a landfill, an oil or concoction spill

(ii) often generally simple to decide mass emanation rates, especially for point sources that are routinely observed for consistence with natural directions

(iii) however, analytical measurement may be difficult due to: trace toxicant concentrations in complex effluent chemical mixtures, difficulties in determination of all relevant reactive metabolites or chemical forms, and temporal fluctuations in toxicant concentrations and flow rates, also many non-regulated/monitored toxicants of potential concern

(iv) often relatively easy to monitor, regulate, and control point source emissions of toxicants into the environment

(v) earliest and most well developed regulatory policy/law and pollution control technologies aimed at point sources

B. Non-point sources – not a discrete, easily identifiable place of entry of chemicals into environment

(i) some examples

- diffuse emissions over widespread geographic areas – farm fields in rural areas, lawns and streets in urban areas
- multiple mobile sources of emissions – vehicles
- multiple, dispersed stationary sources – septic tanks
- atmospheric deposition from point sources and vehicle emissions

(ii) very difficult to accurately determine mass emission rates

(iii) very difficult to monitor, regulate, and control non-point source emissions of toxicants

(iv) current area of concern regarding needed development of regulatory policy/law and pollution control technologies.

8.6 SYNTHETIC ORGANIC COMPOUNDS

The table beneath records some normal working environment materials that meet at least one of the criteria to be viewed as "lethal" and some of potential wellbeing impacts related with

that synthetic. Remember that harmful materials can likewise be found in buyer items that come into the home - dependably read the notice names and safe utilize directions before utilizing any items.

Organic compound	**Potential Health Effects/Symptoms Associated with Toxicity(Note: not a complete listing of symptoms**
Methylene chloride	• Mild central nervous system depressant. May cause headache, nausea, dizziness, drowsiness, in coordination and confusion, unconsciousness and death. • Causes skin and eye irritation.
Isopropyl alcohol (2-propanol)	• Mild central nervous system depressant. High vapour concentrations may cause headache, nausea, dizziness, drowsiness, in coordination, and confusion. Very high exposures may result in unconsciousness and death. • May be irritating to the respiratory tract. • Causes eye irritation. • Swallowing or vomiting of the liquid may cause aspiration (breathing) into the lungs.
Acetone	• Mild central nervous system depressant. • Very high concentrations may cause headache, nausea, dizziness, drowsiness, in coordination and confusion. • Causes eye irritation. • Swallowing or vomiting of the liquid may result in aspiration into the lungs.
l-Limonene	• Causes moderate skin irritation. • May cause allergic skin reaction.
Acetaldehyde	• The vapour is irritating to the respiratory tract. May cause lung

	injury. These effects may be delayed. • Causes severe eye irritation.
Hydrogen peroxide (>35%)	• May be fatal if swallowed.

Note. Reprinted from "Toxic Materials - Hazards: OSH Answers", by Government of Canada, Canadian Centre for Occupational Health and Safety, (2017, October 06). Retrieved from http://www.ccohs.ca/oshanswers/chemicals/Toxic/Toxic_hazards.hTml?wbdisable=true

8.7NATURAL OCCURRING TOXINS

8.7.1 BIOTOXINS

The expression "biotoxin" is here and there used to unequivocally affirm the organic root. Biotoxins are additionally ordered into parasitic biotoxins, or short mycotoxins, microbial biotoxins, plant biotoxins, short phytotoxins and creature biotoxins.

Poisons delivered by microorganisms are vital virulenced eterminants in charge of microbial pathogenicity or potentially avoidance of the host resistant reaction.

Biotoxins change extraordinarily in reason and system, and can be exceptionally intricate (the venom of the cone snail contains many little proteins, each focusing on a particular nerve channel or receptor), or moderately little protein.

Biotoxins in nature have two essential capacities:

- Predation in the bug, wind, scorpion, jellyfish, wasp
- Defense in the honey bee, subterranean insect, termite, bumble bee, wasp, harm dash frog

A portion of the all the more outstanding sorts of biotoxins include:

- Cyanotoxins, created by cyanobacteria
- Dinotoxins, created by Dinoflagellates

- Necrotoxins cause corruption (i.e., demise) in the cells they experience and decimate a wide range of tissue. Necrotoxins spread through the circulation system. In people, skin and muscletissues are most delicate to necrotoxins. Living beings that have necrotoxins include:
    - The dark colored loner or "fiddle back" creepy crawly
    - Most poisonous snakes and snakes deliver phospholipase and different trypsin-like serine proteases
    - Puff Adder
    - Necrotizing fasciitis (the "substance eating" microscopic organisms) - Produces a pore framing poison
- Neurotoxins principally influence the sensory systems of creatures. The gathering neurotoxins for the most part comprise of particle channel poisons that disturb particle channel conductance. Creatures that have neurotoxins include:
    - Theblack widow spider.
    - Most scorpions
    - The box jellyfish
    - Elapid snakes
    - The cone snail
    - The Blue-ringed octopus
    - Venomous angle
    - Frogs
    - Palythoa coral

- Myotoxins are little, essential peptides found in snake and lizard venoms. They cause muscle tissue harm by a non enzymatic receptor based component. Living beings that have myotoxins include:
    - rattlesnakes
    - eastern hairy mythical serpent
- Cytotoxins are dangerous at the level of individual cells, either in a non-particular mold or just in specific sorts of living cells:
    - Ricin, from castor beans
    - Apitoxin, from bumble bees
    - T-2 mycotoxin, from certain harmful mushrooms

8.7.2 INORGANIC CHEMICALS

A. Heavy metals

(i) Lead:

The normally happening component lead was utilized as a part of the Roman period to line vessels and as a ceramics coat, and also in beauty care products. In the nineteenth century in the United States paint makers started to utilize lead as a color, albeit even in 1786, Benjamin Franklin sketched out the unsafe impacts of lead on the body in a letter to Benjamin Vaughan, a companion. Despite the fact that restricted in paint today, society should be ever watchful in ensuring youngsters (who are especially powerless to the impacts of lead on the mind and sensory system) from presentation to more established chipping paint chips. Lead was likewise utilized as a part of gas to anticipate motor thumping. In light of bans of these utilizations and escalated general wellbeing endeavors, lead focus in urban kids has diminished in the previous a very long while. Studies have shown a connection between's insignificant lead presentation and higher intellectual capacity.

(ii) Mercury:

Mercury, as different components in the occasional table at the highest point of this page, does not separate. It happens normally and is found in modest sums in seas, shakes and soil. It moves toward becoming airbourne when rocks separate through disintegration, volcanoes eject and when soil deteriorates. It at that point flows in nature and is redistributed.

The United Nations Environment Program (UNEP) as of late expressed (in 2005) that mercury can be transported in the air and seas around the world a huge number of miles from where it is produced. An UNEP report additionally expresses that coal-let go control stations and waste incinerators directly represent 1,500 tons, or 70%, of new evaluated man-made mercury discharges to the climate. Limitations on contamination from control stations and incinerators could check such discharges. Be that as it may, don't hold your breath!

In 1989, it was evaluated that utilized family unit batteries made up roughly 86% of all dumped mercury, i.e. refined mercury or mercury subordinates that are utilized as a part of mechanical items and after that dumped, regularly in residential waste and not reused or discarded as an exceptional union. Dumped mercury frequently saturates the ground water, dirtying neighbourhood water supplies. The offer of mercury oxide batteries is currently prohibited in a few nations, and confined in different nations. For instance, the USA prohibited mercury oxide batteries with the exception of up to 25mg of mercury for every catch cell battery in the Mercury-Containing and Rechargeable Battery Management Act in 1996.

Different wellsprings of mercury tainting, either from the fabricate of, utilization of or dumping of (for the most part into landfill or modern profluent): research facility reagents and hardware, anodes (e.g. Calomel cathode), thermometers, indicators, dental applications (mercury amalgam fillings), paints, electrical apparatuses, fluctuating diuretics, fluorescent lights, beauty care products, hair colors, the make and conveyance of oil based commodities, and furthermore fungicides and pesticides. In spite of the fact that the utilization in fungicides and pesticides have diminished because of ecological concerns, mercury build-ups still endure in nature (i.e. air, water, soil and so forth.) from past utilize.

A lot of mercury progress toward becoming airbourne when oil, coal, wood and petroleum gas are singed, or when squander containing mercury is burned. Once airbourne, mercury tumbles to the ground with rain and snow, arriving on water or soil, in this manner causing pollution. Lakes and waterways are additionally specifically contaminated by the release of mercury-loaded mechanical and metropolitan waste into them. Once in a waterway, the inorganic mercury (typically Mercuric Chloride) is changed over into natural mercury as methylmercury (CH3Hg) by microscopic organisms through chelation and different procedures (methylation - expansion of a methyl-gathering) - inside sea-going biota and furthermore dregs. Natural mercury is numerous hundred times more poisonous than

inorganic mercury. Fish assimilate the methylmercury from their nourishment source and from the water as it disregards their gills. Mercury is bound firmly to proteins in all tissues in angle. For instance, underneath is an by Daphine Zuniga about her mercury harming from a high fish consume less calories.

As per Doctor's Data (Urine Toxic Metals Test Report), in light of different scholarly reference sources, the human admission of dietary mercury (particularly) is unimportant (except for angle), unless the sustenance is sullied with one of the above sources. An everyday eating regimen of fish can bring about 1-10 micrograms of mercury to be ingested every day, with around 3/4 of this as methyl mercury.

Inorganic mercury (mercuric salts or mercuric oxide) is changed over into natural mercury, either methyl mercury or ethyl mercury (C2H5Hg) in the gastro intestinal tract by microbes where it can be all the more promptly consumed by the tissues including the cerebrum. On the off chance that amalgam fillings are releasing, the mercury they emanate is inorganic. This mercury tends to develop in the internal organ where it is changed over to natural mercury. More data on amalgam fillings can be found in the Mercury Amalgam Fillings area.

'One of the significant issues with substantial metals, particularly mercury, is the high level of reabsorption through the gut. While natural mercury is ineffectively assimilated through the gut, its natural methylated shape is exceedingly ingested (90-95% retention). The non-natural basic mercury discharged through the bile is frequently methylated by unusual microscopic organisms or yeast in the gut bringing about re-assimilation of methylated mercury. Natural mercury (from fish and fish) and basic mercury assimilated from different poisons can likewise be retained through the stomach related tract. The methylated mercury can pass the blood-mind boundary, and apply its dangerous impacts in the cerebrum either as methylated mercury, or in the wake of being demethylated in the cerebrum, as essential mercury.'

Paul Cutler contends that both inorganic and natural mercury can be destructive, just in various ways. Natural mercury isn't hurtful to the kidneys yet effectively crosses the blood cerebrum boundary. Inorganic mercury is substantially more destructive however can't without much of a stretch cross the blood cerebrum barrer. The half existence of transformation of natural to inrganic mercury is around 44 days. It is assessed that roughly 5-10% of the aggregate sum of natural mercury expended winds up in the mind as inorganic mercury (i.e. changing over from natural to inorganic shape which does the real harm).

Inorganic mercury is inadequately consumed by the stomach related tract, and the liver and gallbladder discharge mercury from the body in its inorganic frame.

It is felt that the noteworthy overwhelming metal levels exhibit in certain fish species implies that the individuals who bolster frequently on angle, i.e. chickens and their eggs (and a few people) might be higher in their mercury fixations.

Mercury is really 13 times heavier than water, yet isn't really 'wet'. Press objects glide over mercury.

(iii)Cadmium:

Cadmium gets from delicate water, tobacco smoke, air contamination, fish, teas, bone supper, oxide cleans, paints and welding and so on. Cadmium alongside lead, mercury and arsenic is a delicate Lewis corrosive, with specific proclivity for delicate Lewis bases, for example, the sulphydryl side chain of cysteine amino acids. Therefore it is conceivable that the substantial metals apply their harmful impacts by holding with basic cystein deposits in proteins; in spite of the fact that the real physiological outcomes differ starting with one metal then onto the next.

The substance properties of cadmium are considerably nearer to zinc than mercury. The principle hotspots for cadmium in the earth are from coal, zinc mining, refining of metals and tobacco smoking. The cadmium work in rural soils is of concern. These cadmium contributions to soils are chiefly from air borne affidavit from business phosphate manures, which contain cadmium as a characteristic constituent of phosphate mineral. The cadmium focus would additionally increment with the utilization of compost from sewage ooze (which is regularly defiled with cadmium and different metals). Soil conditions were absolutely a factor in the main known instance of far reaching ecological cadmium harming, which occured in the Jinzu valley of Japan. Water system water drawn from a stream that was polluted by zinc mining and refining complex prompted elevated amounts of cadmium in rice. Several individuals in the range created degenerative bone sickness called itai-itai because of impedance of cadmium with Ca2+ testimony. Their bones wound up noticeably permeable and subject to fall. Incessant presentation to cadmium has been connected to heart and lung malady, insusceptible concealment, and liver and kidney illness. Cadmium assaults the dynamic destinations of protein hindering basic capacity. The protein repressed by Cd2+ incorporates adenosine triphosphate, liquor dehydrogenase, anylase, carbonic anhydrase,

peptidase action in carboxy peptidase and glutamic oxaloacetic transminase. As said the Cd2+ requestering protein metallothionen gives assurance until the point that its ability is surpassed. Since metallothionen is amassed in the kidney, this organ is harmed first by inordinate cadmium. Whatever remains of the cadmium is put away in the body and gathers with age. At the point when an over the top measure of Cd2+ is ingested, it replaces Zn2+ at key enzymatic destinations causing metabolic clutters.

B. Oxides of nitrogen and sulphur:

Nitrous oxide (N2O) is utilized as an oxidant gas and in dental surgery as a general soporific. It is a focal sensory system depressant and can go about as asphyxiant.Nitric oxide (NO) and nitrogen dioxide (NO2) are the two noteworthy oxides of nitrogen which influence human wellbeing. NO, as it is does not demonstrate any unfavorable wellbeing impacts. In any case, it winds up noticeably lethal when it is oxidized to NO2. NO2, after inward breath achieves the dampness filled alveoli of the lungs. There it is changed over into nitrous corrosive and nitric corrosive which are very disturbing and make harm the lung tissues. Biochemically NO2 disturbs lactic dehydrogenase and some other protein frameworks. Free radicals especially HO• are likely framed in the body by the activity of NO2 and the compound presumably causes lipid peroxidation in which the C=C twofold securities in unsaturated body lipids are assaulted by free radicals and experience chain response within the sight of O2 bringing about their oxidative pulverization. NO2 in blend with hydrocarbons goes about as the initiator of photochemical exhaust cloud prompting the generation of optional contaminations like the oxidants. These oxidants are the ones that reason harm to human wellbeing.

The principle worry of SO2 in urban environments emerge not from SO2, but rather from the progressions it experiences in the climate, for example, the development of H2SO4 and sulfate pressurized canned products. The sulfate particles can be conveyed profound into the lungs, causing significantly more extreme medical issues. SO2 can likewise be assimilated on little particulates, for example, the salts of iron, manganese and vanadium exhibit in the environment and accordingly enter the alveoli. There within the sight of wet air, SO2 is oxidized to H2SO4 and the particulates go about as impetuses in improving the oxidation procedure.

C. Asbestos:

"Asbestos" is a mechanical term for various hydrated silicates with an inexact equation Mg3 P(Si2O5) (OH)4. They isolate into solid adaptable strands after pulverizing and preparing. Inward breath of asbestos tidy or strands can cause a crippling lung sickness known as asbestosis. The infection is portrayed by shortness of expansiveness and pleural calcification. Asbestos has additionally been demonstrated to actuate lung disease. The strands line the layers of the lungs and guts and this can prompt mesothelioma, a hopeless and lethal malignancy.

8.8 DOSE-RESPONSE RELATIONSHIP

The measurements impact relationship is the connection amongst dosage and impact on the individual level. An expansion in measurements may build the power of an impact, or a more serious impact may come about. A measurement impact bend might be acquired at the level of the entire living being, the cell or the objective particle. Some harmful impacts, for example, passing or growth, are not reviewed but rather are "all or none" impacts.

The dosage reaction relationship is the connection amongst measurements and the level of people demonstrating a particular impact. With expanding dosage a more prominent number of people in the uncovered populace will for the most part be influenced.

It is basic to toxicology to set up dosage impact and measurement reaction connections. In therapeutic (epidemiological) thinks about a rule regularly utilized for tolerating a causal connection between an operator and an infection is that impact or reaction is relative to dosage.

A few dosage reaction bends can be drawn for a synthetic—one for each sort of impact. The measurement reaction bend for most dangerous impacts (when contemplated in vast populaces) has a sigmoid shape. There is generally a low-dosage go where there is no reaction identified; as measurement builds, the reaction takes after a climbing bend that will for the most part achieve a level at a 100% reaction. The dosage reaction bend mirrors the varieties among people in a populace. The incline of the bend changes from concoction to synthetic and between various sorts of impacts. For a few chemicals with particular impacts (cancer-causing agents, initiators, mutagens) the measurement reaction bend may be straight from dosage zero inside a specific measurements go. This implies no edge exists and that even little measurements speak to a hazard. Over that measurements go, the hazard may increment at more prominent than a straight rate.

Variety in presentation amid the day and the aggregate length of introduction amid one's lifetime might be as vital for the result (reaction) as mean or normal or even coordinated measurements level. High pinnacle exposures might be more destructive than an all the more even presentation level. This is the situation for some natural solvents. Then again, for a few cancer-causing agents, it has been tentatively demonstrated that the fractionation of a solitary dosage into a few exposures with a similar aggregate measurement might be more powerful in delivering tumors.

A dosage is frequently communicated as the measure of a xenobiotic entering a living being (in units, for example, mg/kg body weight). The measurements might be communicated in various (pretty much educational) ways: introduction dosage, which is the air centralization of toxin breathed in amid a specific day and age (in work cleanliness normally eight hours), or the held or consumed measurements (in modern cleanliness additionally called the body load), which is the sum show in the body at a specific time amid or after presentation. The tissue measurements is the measure of substance in a particular tissue and the objective dosage is the measure of substance (for the most part a metabolite) bound to the basic particle. The objective dosage can be communicated as mg substance bound per mg of a particular macromolecule in the tissue. To apply this idea, data on the instrument of harmful activity on the sub-atomic level is required. The objective measurements is all the more precisely connected with the lethal impact. The introduction measurements or body weight might be all the more effortlessly accessible, however these are less accurately identified with the impact.

In the measurement idea a period viewpoint is frequently included, regardless of the possibility that it isn't generally communicated. The hypothetical dosage as indicated by Haber's law is D = ct, where D is measurements, c is grouping of the xenobiotic noticeable all around and t the span of introduction to the synthetic. In the event that this idea is utilized at the objective organ or sub-atomic level, the sum per mg tissue or particle over a specific time might be utilized. The time perspective is generally more imperative for understanding rehashed exposures and ceaseless impacts than for single exposures and intense impacts.

Added substance impacts happen because of presentation to a mix of chemicals, where the individual toxicities are just added to each other (1+1=2). At the point when chemicals act through a similar system, additivity of their belongings is accepted despite the fact that not generally the situation in actuality. Connection between chemicals may bring about a restraint

(enmity), with a littler impact than that normal from expansion of the impacts of the individual chemicals (1+1<2). Then again, a mix of chemicals may create a more articulated impact than would be normal by expansion (expanded reaction among people or an expansion in recurrence of reaction in a populace), this is called synergism (1+1>2).

Inactivity time is the time between first introduction and the presence of a recognizable impact or reaction. The term is frequently utilized for cancer-causing impacts, where tumors may show up quite a while after the begin of presentation and at times long after the end of introduction.

A dosage edge is a measurement level beneath which no discernible impact happens. Edges are thought to exist for specific impacts, similar to intense poisonous impacts; yet for nobody else, as cancer-causing impacts (by DNA-adduct-framing initiators). The unimportant nonappearance of a reaction in a given populace ought not, be that as it may, be taken as proof for the presence of a limit. Nonattendance of reaction could be because of basic factual marvels: an unfriendly impact happening at low recurrence may not be discernible in a little populace.

LD50 (viable measurements) is the dosage causing half lethality in a creature populace. The LD50 is regularly given in more established writing as a measure of intense poisonous quality of chemicals. The higher the LD50, the lower is the intense lethality. An exceptionally dangerous synthetic (with a low LD50) is said to be strong. There is no fundamental relationship amongst's intense and incessant poisonous quality. ED50 (successful measurements) is the dosage causing a particular impact other than lethality in half of the creatures.

NOEL (NOAEL) implies the no watched (unfavorable) impact level, or the most astounding measurements that does not cause a poisonous impact. To build up a NOEL requires numerous measurements, an extensive populace and extra data to ensure that nonattendance of a reaction isn't simply a factual marvel. LOEL is the most reduced watched powerful measurements on a dosage reaction bend, or the least measurement that causes an impact.

A wellbeing factor is a formal, discretionary number with which one partitions the NOEL or LOEL got from creature examinations to get a provisional admissible measurements for people. This is frequently utilized as a part of the zone of nourishment toxicology, however might be utilized likewise in word related toxicology. A security factor may likewise be

utilized for extrapolation of information from little populaces to bigger populaces. Wellbeing factors go from 100 to 103. A wellbeing element of two may ordinarily be adequate to shield from a less genuine impact, (for example, disturbance) and a factor as vast as 1,000 might be utilized for intense impacts, (for example, growth). The term wellbeing element could be better supplanted by the term insurance factor or, even, vulnerability factor. The utilization of the last term reflects logical vulnerabilities, for example, regardless of whether correct measurement reaction information can be made an interpretation of from creatures to people for the specific compound, poisonous impact or presentation circumstance.

Extrapolations are hypothetical subjective or quantitative evaluations of poisonous quality (hazard extrapolations) got from interpretation of information starting with one animal types then onto the next or from one arrangement of dosage reaction information (ordinarily in the high measurements run) to locales of dosage reaction where no information exist. Extrapolations generally should be made to anticipate harmful reactions outside the perception go. Numerical displaying is utilized for extrapolations in view of a comprehension of the conduct of the synthetic in the creature (toxicokinetic demonstrating) or in light of the comprehension of likelihood that particular organic occasions will happen (organically or unthinkingly based models). Some national offices have created advanced extrapolation models as a formalized strategy to foresee dangers for administrative purposes.

8.9 ROUT OF ENTERY

Dangerous materials are substances that may make hurt an individual on the off chance that it enters the body. Dangerous materials may enter the body in various ways. These ways are known as the course of presentation. The most widely recognized course of presentation is through inward breath (breathing it into the lungs). Another basic course of section is through skin contact. A few materials can without much of a stretch go through unprotected skin and enter the body. Ingestion is another, less normal, course of presentation in the work environment. Ingestion frequently happens incidentally through poor cleanliness hones (e.g. eating sustenance or smoking a cigarette utilizing debased hands).

8.10 ENVIRONMENTAL MOVEMENT

The physical (non-natural) development of discharged chemicals will decide spatial and fleeting appropriation in the earth:

- medium is generally air or water
- toxins frame in travel might be vaporous, disintegrated or particulate
- two significant types of physical transport are shift in weather conditions and dissemination

A. Advection – development of the vehicle medium, concoction toxicant basically "comes for the ride" with the mass development of the medium

- two sorts, homogeneous shift in weather conditions and heterogeneous shift in weather conditions

(i) Homogeneous shift in weather conditions - development in a solitary transport medium

- examples – transport of concoction in air on a blustery day or transport of a synthetic disintegrated in water in a streaming stream
- advective streams frequently overwhelm compound transport in the environment and amphibian and marine frameworks
- advective air and water streams additionally happen in soil frameworks, yet the rate of development is extensively slower than in the environment or waterways

(ii) Heterogeneous shift in weather conditions – (at least two) transport media are associated with synthetic development

- examples - substance transported in air experiences barometrical synthetic statement into water or soil and is additionally transported in the second medium or concoction
- transport in water experiences sorption to suspended particles and settles to base to be additionally transported by residue development
- dynamics of heterogeneous transport are more mind boggling than flow of homogeneous transport

B. Diffusion – development of the substance toxicant itself, development is from an area of moderately high fixation to a position of lower focus

- may happen inside a medium (or stage) or between media (or stages)

(i) Diffusion inside a stage or medium

- may happen by arbitrary (warm) movement of the compound (sub-atomic dissemination), by irregular turbulent blending of the vehicle medium (turbulent dispersion), or by a mix of both

(ii) Diffusion between stages or media

- occurs at interface between two media, air-water, molecule water, or organic film water
- diffusive transport will drive a substance between media until the point when the balance fixations are come to in each stage

8.11 FATE OF TOXINS

Poisons can cause genuine wellbeing impacts in an uncovered person. The level of destiny, related with any poison is identified with the correct, centralization of the poison, the course into the body and the sum consumed by the body (thee measurements). The weakness of individual likewise assumes a critical part. Remember that poison specialists can have other destiny related with it. For instance, a poison operator may likewise be destructive and combustible.

8.12 MODE OF ACTIONS

Poisons can influence the wellbeing through two unique modes one could be quickly impact or deferred. Wellbeing impacts that happen promptly after a solitary presentation are called intense impacts. In different cases, wellbeing impacts won't happen until some point after the presentation. This is known as an incessant impact. An incessant impact may happen hours, days, months or even a long time after presentation. By and large, intense impacts are caused by a solitary, generally high introduction. Ceaseless impacts have a tendency to happen over a more drawn out timeframe and include bring down exposures (e.g., introduction to a littler sum after some time). Some dangerous materials can have both intense and ceaseless wellbeing impacts.

8.13 NATURAL POISONS

Numerous harmful chemicals are available normally in the earth. For instance, all of metals and different components are far reaching in the earth, however under a few conditions they may happen normally in focuses that are sufficiently huge to be toxic to in any event a few life forms.

Example of common "contamination" can include surface presentation of minerals containing substantial groupings of lethal components, for example, copper, lead, selenium, or arsenic. For instance, soils impacted by a mineral known as serpentine can have huge groupings of

dangerous nickel and cobalt, and can be harmful to generally plants. In different cases, certain plants may specifically take up components from their condition, to the extent that their foliage turns out to be intensely poisonous to herbivorous creatures. For instance, soils in semi-parched areas of the western United States regularly contain selenium. This component can be bio accumulated by specific types of **legumes** known as locoweeds (Astragalus spp.), to the extent that the plants turn out to be greatly noxious to cows and to other expansive creatures that may eat their dangerous foliage.

In a few conditions, the nearby condition can turn out to be normally contaminated by gasses at harmful fixations, harming plants and creatures. This can occur in the region of volcanoes, where vents known as fumaroles regularly radiate lethal sufur dioxide, which can toxin and murder adjacent plants. The sulfur dioxide can likewise dry-store to the close-by ground and surface water, causing a serious fermentation, which brings about solvent aluminium particles getting to be noticeably poisonous.

Other normally happening poisons are biochemical's that are combined by plants and creatures, frequently as an impediment to herbivores and predators, individually. Truth be told, probably the most dangerous chemicals known to science are biochemical's orchestrated by creatures. One such case is tetrodotoxin, blended by the Japanese globe fish (Spheroides rubripes), and to a great degree dangerous regardless of the possibility that ingested in modest sums. Just somewhat less harmful is saxitoxin, blended by types of marine phytoplankton, however collected by shellfish. At the point when individuals eat these shellfish, a dangerous disorder known as disabled shellfish harming comes about. There are various different cases of fatal biochemical, for example, snake and honey bee venoms, poisons created by pathogenic microorganisms, and mushroom harms.

8.13.1 SELENIUM IN GRAIN

Selenium (Se) enters the natural way of life by means of plant and microorganism change of inorganic selenium to naturally bound structures. Selenium poisonous quality (i.e., selenosis), caused by inordinate selenium admission, has happened on a huge scale in seleniferous districts in China as the after-effect of expanded utilization of selenium-containing sustenances (inexact day by day admission of 3– 6.5 mg Se/day). The most well-known indications of selenosis are loss of hair, distortion, and loss of nails. Other detailed side effects incorporate expanded blood selenium levels, the runs, exhaustion, a garlic-like scent

of the breath and real emissions, touchiness, fringe neuropathy, and skin injuries. Selenium consumption levels that reason selenosis have not yet been all around characterized. Concentrates in China recommend that around 3– 5 mg/day (0.05– 0.08 mg/kg/day)will cause selenosis. Inhabitants of seleniferous areas in South Dakota who devoured roughly 700 µg selenium/day (0.01 mg/kg/day) demonstrated no indications of selenosis. The EPA has proposed an oral reference measurement (RfD) of 0.005 mg/kg bw/day, or 350 µg/day.

8.13.2 METHYL MERCURY IN SEAFOOD

Introduction to natural mercury is generally uncommon, in spite of the fact that was before a word related infection of cap makers as essential mercury was utilized for the curing of creature pelts. Inward breath of the mercury exhaust prompted mental weakening and in this manner named ―mad hatter syndrome‖. Important to nourishment toxicology, is the methyl subordinate, methyl mercury, framed by bacterial activity in a sea-going condition from anthropogenic and normal wellsprings of basic mercury. Anthropogenic sources incorporate consuming of coal (which contains mercury), chloralkali process and different wellsprings of natural mercury into sea-going situations. On account of Minamata, Japan, there was an immediate release of methyl mercury into the earth. Methyl mercury presentation may cause neurological paresthesias, ataxia, and dysarthria, hearing deformities and passing. Formative postponements have been archived in youngsters borne of moms presented to methyl mercury. Other than guide presentation to methyl mercury, introduction typically occurs as the aftereffect of methyl mercury getting to be plainly fused into the evolved way of life, climbing as every predator expends the littler and less blessed creature. Close to the pinnacle of the natural way of life, methyl mercury winds up plainly amassed in angle including, bonito (Sarda spp.), halibut (Hippoglossus spp.), mackerel (Scomberomorus spp.), marlin (Makaira spp.), shark (all species), swordfish (Xiphias gladius), and bluefin fish (Thunnus spp.). The determination of these species depended on chronicled information on levels of methyl mercury found in angle expended in the U.S. The choice was additionally in view of a FDA activity level of 1.0 ppm in the palatable bit of fish. In any case, the reasonable level of mercury relies upon whether the mercury was ―added‖; that is, did the nearness of mercury emerge from an anthropogenic source (i.e., was the fish got in a region known for mercury release), or was not included and the after effect of mercury normally show in nature.

8.13.3 PRUSSIC ACID IN CHERRY, APPLE AND PEACH PITS

Prussic corrosive (otherwise called hydrocyanic corrosive, hydrogen cyanide, or cyanide) is shaped when cyanogenic glycosides found in leaves, cherry, apple and peach pits, oak greenery and other plant tissues are harmed and come into contact with beta-glycosidase or emulsion proteins. The proteins discharge the cyanide from the glycoside, and the cyanide keeps the body's cells from using oxygen, bringing about cell putrefaction and tissue harm. The mucous layers and blood are brilliant red as they are oxygenated; however the cells in the tissues can't use the oxygen. Clinical indications of prussic corrosive harming incorporate quick breathing, trembling, incoordination and in outrageous cases, respiratory as well as heart failure. Many natural product trees contain prussic corrosive glycosides in the leaves and seeds, yet just immaterial levels are available in the beefy parts of the organic product. In the west African tropics, cassava is expended as a dietary staple and wrong treatment of the cassava before handling and utilization can bring about a constant type of cyanide harming named "tropical ataxic neuropathy", the after effect of demyelinization of the optic, sound-related, and fringe nerve tracts.

Prussic corrosive as found in enhancing fixings is restricted to 25 ppm in cherry pits (Prunus avium L. or, on the other hand P. cerasus L.), cherry shrub leaves (Prunus laurocerasus L.), senior tree leaves (Sambucus nigra L.), and peach leaves (Prunus persica (L.) Batsch) (21 CFR 172.510); despite the fact that the concentrate of intense almond (Prunus amygdalus Batsch, Prunus armeniaca L., or Prunus persica (L.) Batsch) must be prussic corrosive free (21 CFR 182.20). There are no FDA controls or rules limiting the nearness of prussic corrosive in apple seed (Malus spp.), likely on the grounds that concentrates of these seeds have no financial incentive as flavor fixings.

8.13.4 HYPERICIN IN ST. JOHN'S WORT

St. John's wort (Hypericum perforatum; Figure 1) is a natural idea to lighten indications of despondency, and institutionalized concentrates of St. John's wort are devoured commonly in tablet or container shape. The real dynamic upper constituents in St. John's wort are believed to be hyperforin and hypericin. The component of activity isn't completely seen, yet may include restraint of serotonin (5-HT) reuptake, like regular upper medications. In this way, hyperforin and hypericin brought in conjunction with other serotonin reuptake inhibitors may add to serotonin disorder, a conceivably hazardous rise of serotonin in the focal sensory system. Hyperforin is additionally known to instigate cytochrome P450 proteins CYP3A4 and CYP2C9, which can prompt expanded digestion of specific medications and diminished clinical reaction.

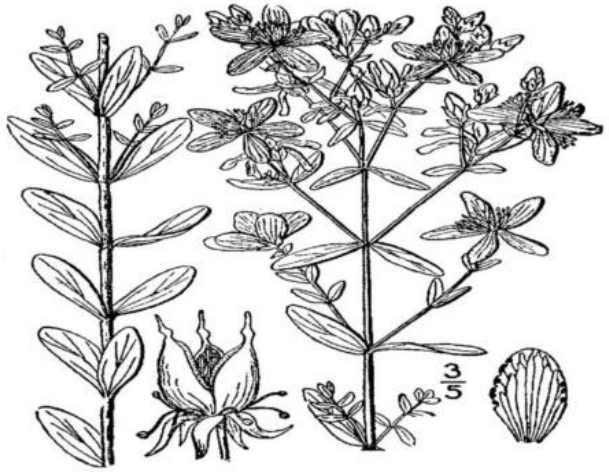


*Figure: 8.1 St. John's wort (*Hypericum perforatum*)*

In substantial measurements, St. John's wort is toxic to brushing creatures, with distributed instances of domesticated animals harming described by general fretfulness and skin bothering, hind limb shortcoming, gasping, perplexity, misery and in a few occurrences, insanity and hyperactivity bringing about the creature running in hovers until depleted. In people, utilization of St. John's wort may bring about photosensitization, and at high ceaseless measurements, some liver harm may happen. As far as possible presentation to St. Johns wort (Hypericum perforatum), including the leaves, blooms, and caulis, by ordering that lone without hypericin liquor distillate shape might be utilized and afterward, just in mixed refreshments (21 CFR 172.510).

8.13.5 GOITROGENS (GLUCOSINOLATES) IN BRASSICASPP

Certain crude sustenances have been found to contain substances that stifle the capacity of the thyroid organ by meddling with the take-up of iodine, a fundamental supplement in development, intellectual capacity, and hormonal adjust. An absence of useful iodine is known to bring about psychological insufficiencies (e.g., Cretinism). The decline in iodine take-up makes the thyroid organ broaden, shaping a goiter. Sustenances that have been distinguished as goitrogenic incorporate spinach, cassava, peanuts, soybeans, strawberries, sweet potatoes, peaches, pears, and vegetables in the Brassica sort, which incorporate broccoli, brussels grows, cabbage, canola, cauliflower, mustard greens, radishes, and rapeseed. Goiter has additionally been ascribed to the utilization of vast amounts of uncooked kale or cabbage.

High temperatures (i.e., cooking) inactivate the goitrogenic substances, on the whole named glucosinolates. Cassava (Manihot esculenta) is a basic dietary wellspring of vitality in the tropics, however contains abnormal amounts of linamarin, a glucosinolate. Cassava must be appropriately prepared dried, absorbed water or heated to adequately decrease the linamarin . Glucosinolates are sulfur-containing substances that are utilized in the body by thioglucosidase to shape thiocyanate, isothiocyanate, nitriles and sulfur. Under specific conditions the isothiocyanates experience cyclization to frame goitrins, expanding their powerful goitrogenic action. The oils from rapeseed (Brassica napus) must be investigated for potential goitrins to evade potential goitrogenic action while expending these oils. No FDA directions were situated for reasonable convergences of glucosinolates in human nourishment. Glucosinolates (figured as epi-progoitrin) and goitrin are restricted to not over 4% and 0.1% (individually) of the seed supper of Crambe abyssinica (Crambe feast) acquired after the evacuation of the oil and utilized as a creature sustain fixing (21 CFR 573.310).

8.13.6 ERUCIC ACID IN RAPE

Assault (Brassica napus L. or, on the other hand Brassica campestris L.) is a yearly herb of the mustard family local to Europe and is developed in the United States since it produces oil-rich seeds for cooking oil. Rapeseed oil had been utilized for a long time as oil for lights and all the more as of late as machine oil grease. Far reaching utilization of rapeseed oil as a sustenance fixing was not considered until the late 50s. Be that as it may, early examinations found that sustaining abnormal amounts of rapeseed oil to rats fundamentally expanded

cholesterol levels in the adrenal organs and lipidosis in the cardiovascular tissue. This impact was additionally noted in chickens, ducks and turkeys nourished abnormal amounts of rapeseed oil, bringing about development impediment, mortality, and a thickening of the epicardium and expanded stringy tissue in various regions of the myocardium. Erucic corrosive was distinguished as the causative specialist of these impacts of rapeseed oil. Erucic corrosive is a long-chain unsaturated fat with one unsaturated carbon-carbon security (C22:1). Abnormal amounts of erucic corrosive have been gotten a kick out of the chance to greasy store arrangement in heart muscle in creatures. Erucic corrosive is ineffectively oxidized by the mitochondrial β-oxidation framework, particularly by the myocardial cells, which brings about a gathering of erucic corrosive, creating myocardial lipidosis which has been accounted for to lessen the contractile power of the heart. Albeit myocardial lipidosis due to erucic corrosive utilization has not been affirmed in people, creature bolstering contemplates affirmed the development of myocardial lipidosis in an assortment of creature animal types in a measurements subordinate way, which has been the standard appraisal by government organizations of potential unfavorable impacts in people. Canola oil is acquired from Canola (Canadian oil, low corrosive), a rapeseed assortment that was ordinarily reared in the late 1970s in Canada to contain decreased levels of erucic corrosive and glucosinolates. As far as possible the measure of erucic corrosive in Canola oil to close to 2% of the part unsaturated fats (21 CFR 184.1555).

8.14 SUMMARY

Wellbeing experts require access to ecological wellbeing and toxicology data for some reasons. Absolutely, open mindfulness about human wellbeing dangers from synthetic and biologic specialists in the earth has expanded significantly as of late. Correspondingly, changing patterns in medicinal services and an accentuation on counteractive action, combined with expanding PC education, all help the requirement for promptly accessible data about the effects of unsafe substances in nature on individual and general wellbeing. Reports in the famous press and news media have featured people in general's worry. For instance, pesticides on sustenances; second-hand tobacco smoke; asbestos and lead paint in homes and open structures; dioxin sullying; word related exposures to gas and different chemicals; presentation to radon and benzene; and drinking water debased with biologic or concoction operators are only a couple of the issues that may defy the American open.

Despite the fact that the general population depends intensely on government and state administrative offices for insurance from exposures to unsafe substances, they oftentimes seek wellbeing experts for data on courses of introduction and the nature and degree of related antagonistic wellbeing results. Be that as it may, most wellbeing experts secure just a negligible learning of toxicology amid their instruction and preparing. Therefore, their working learning of the unfavorable impacts of chemicals on wellbeing and the conditions under which those impacts may happen is frequently restricted. Besides, with the many contending requests on wellbeing experts' chance, it is troublesome, notwithstanding for pros, to keep advised of quickly advancing toxicology data. Hence, wellbeing experts require prepared access to toxicology and ecological wellbeing data assets to help them with persistent care. Policymakers, wellbeing consultants, analysts, wellbeing instructors, and the overall population likewise require access to this data as they seek after their own particular request.

The Toxic Substances Control Act of 1976 furnishes EPA with specialist to require detailing, record-keeping and testing necessities, and confinements identifying with synthetic substances as well as blends. Certain substances are for the most part prohibited from TSCA, including, among others, sustenance, medications, beautifying agents and pesticides.

8.15 GLOSSARY

Abortifacient: Substance that causes pregnancy to end prematurely and causes an abortion.

Absolute lethal concentration (LC100): Lowest concentration of a substance in an environmental medium which kills 100 % of test organisms or species under defined conditions.

Note: This value is dependent on the number of organisms used in its assessment.

Absorbed dose (of a substance): internal dose Amount (of a substance) taken up by an organism or into organs or tissues of interest.

Absorbed dose (of ionizing radiation), D: Energy imparted by ionizing radiation to a specified volume of matter divided by the mass of that volume.

Acaricide: Substance intended to kill mites, ticks, or other Acaridae.

Chain of custody - ensuring evidence is secure and traceable at all times.

Chromatographic techniques - analytical procedure used for separation of compounds or drugs.

Derivitization - process of modifying original compound/drug for enhanced analytical detection.

Drug or toxin concentration - the amount of drug, metabolite or other toxin in a given volume of plasma, urine, other fluid, tissue homogenate, etc.(e.g., number of micrograms, nanograms or picograms per millilitre).

Forensic practitioner - A person, usually police officer, scientist or physician who is engaged in forensic investigations.

Hazard - the biological effects produced by substances (i.e., toxicity). Hazards pose risks only if the exposure is sufficiently high.

Histalogical - pertaining to the minute structure of animal and plant tissues as discernible with the microscope.

Homogenization - process of preparing tissue for analysis by grinding a known amount of tissue in a known amount of water.

Immunoassays – a biochemical test that measures the presence or concentration of a substance in solutions that frequently contain a complex mixture of substances.

Medico-legal death investigation (MDLI) - A medical investigation performed by especially trained forensic medical practitioners, often in conjunction with forensic scientists, to determine the cause and manner of death.

Metabolism - the sum of the processes by which a particular substance is handled in the living body.

Metabolite - a product of metabolism.

Poison - a substance that through its chemical action usually kills, injures, or impairs an organism.

Post-mortem redistribution - recognized toxicological phenomenon of an increase in drug concentration after death.

Specimens - Biological samples collected from a living or deceased person that can be analysed for one or more substances relevant to the matter.

Toxic - containing or being poisonous material especially when capable of causing death or serious debilitation.

Toxicant - a toxic agent.

Toxicity - the biological effect of a substance. In this context, toxicity and hazard are used interchangeably.

Toxicology - a science that deals with poisons and their effect and with the problems involved (as clinical, industrial, or legal).

8.16 SELF ASSESSMENT QUESTIONS AND POSSIBLE ANSWERS

Multiple choice Questions:

1. Phosphine liberated in the stomach in aluminum phosphide poisoning is toxic to all except (Ans-B)
   a. Lungs b. luminium
   c. Liver d. Heart
2. Paraquat poisoning causes (Ans-D)
   a. Renal failure b. Cardiac failure
   c. Respiratory failure d. Multiple organ failure
3. Ecstasy toxicity causes (Ans-E)
   a. Hypereflexia b. Trismus
   c. Dilated pupils d. Visual hallucinations
   e.All of the above
4. The most useful bedside test to suggest snake bite envenomation is (Ans-B)
   a. Prothrombin time b. 20 min whole blood clotting time
   c. International normalized ratio d. Platelet count
5. All substances can be toxic depending on the dosage
   a. True b. False
   c. Wrong d. Not it

Fill in the blanks

1. Yu-cheng Disease in Taiwan is due to the toxic effect of _____ .

a. lead **b. PCBs**

c. dioxin d. asbestos

e. mercury

2. _____is the most likely process of absorption for amino acids.

a. diffusion **b. facilitated diffusion**

c. active transport d. endocytosis

3. The largest percent of antibodies belong to the _____ class.

a. IgG b. IgE

xc c. IgM d. IgA

4. Absorption of the poison is more by_____

a. inhalation b. ingestion

c. Skin contact d. Absorption is similar in all routes

5. The source of poison that give least frequent number of casesbut most serious is_____

a. Animal source. b. Plant source.

c. Chemical source.

d. All of the above give almost the same frequency of cases.

Short Questions:

1. What are the possible toxic mechanisms for chemicals?
2. List the variety of processes of absorption including their characteristics.
3. How do toxic substances enter the body?
4. What are the major functions of the skin? The skin can help to :
5. What are the three major mechanisms for the harmful effects of environmental toxins?
6. List the four major types of hypersensitivity reactions:

Long Questions:

1. Describe different kind of toxicants and their sources?
2. Give brief details about dose-response relationship of toxins?
3. What are the routes of entry of toxic substances into human body?
4. Define the environmental movement and fate of toxins?
5. What are the chronic modes of action?
6. List out the natural poisons?

8.17 REFERENCES

1. Carrington C., Bolger M. An Exposure Assessment for Methylmercury from Seafood for Consumers in the United States. [(Accessed on 21 July 2010)]. Available online: http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/Seafood/FoodbornePathogensContaminants/Methylmercury/UCM114740.pdf. [PubMed].

2. European Commission. Scientific Committee on Food Opinion of the scientific committee on food on thujone. Feb 6, 2003. [(Accessed on 21 July 2010)]. Available online: http://ec.europa.eu/food/fs/sc/scf/out162_en.pdf.

3. Hutt P.B., Merrill R.A., Grossman L.W. Food and Drug Law.3rd. Foundation Press; New York, NY, USA: 2007. p. 369.

4. Matsumura, Y. and Ananthaswamy, H.N. (2004): "Toxic effects of ultraviolet radiation on the skin". Toxicology and Applied Pharmacology. 195: 298-308.

5. Reilly C. Selenium: Physiology, dietary sources and requirements. In: Sadler M.J., editor. Encyclopaedia of Human Nutrition. Academic; San Diego, CA, USA: 1998. pp. 1752–1758.

6. Sors T.G., Ellis D.R., Salt D.E. Selenium uptake, translocation, assimilation and metabolic fate in plants. Photosynth. Res.2005; 86:373–389. [PubMed].

7. United States Environmental Protection Agency (EPA) Selenium and compounds (CASRN 7782-49-2) Mar 1, 1991. [(Accessed on 21 July 2010)]. Available online: http://www.epa.gov/iris/subst/0472.htm.

8. United States Food and Drug Administration (FDA) Chapter 10: Methyl Mercury. Fish and Fisheries Products Hazards and Controls Guidance. 3rd. Jun, 2001. [(Accessed on 21 July 2010)]. Available online: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/ucm092041.htm.

9. Waldron H.A. Did the Mad Hatter have mercury poisoning? Br. Med. J. 1983; 287:1961. Doi: 10.1136/bmj.287.6409.1961.[PMC free article] [PubMed] [Cross Ref].

10. Yang G., Wang S., Zhou R., Sun S. Endemic selenium intoxication of humans in China. Am. J. Clin. Nutr. 1983; 37:872–881. [PubMed]

UNIT 9: ANALYTICAL TOXICOLOGY

CONTENTS

9.1 OBJECTIVES

The study of this unit will let students to:

- Define Analytical toxicology
- Describes the toxic responses of blood
- Explain Organ function tests

9.2 INTRODUCTION

Investigative toxicology is the location, distinguishing proof, and estimation of remote mixes (xenobiotics) in natural and different examples. Investigative techniques are accessible for an extensive variety of intensifies: these might be chemicals, pesticides, pharmaceuticals, medications of mishandle and normal poisons.

Scientific toxicology can aid the analysis, administration, forecast, and anticipation of harming. What's more explanatory toxicology research facilities might be engaged with a scope of different exercises, for example, the evaluation of presentation following concoction episodes, restorative medication checking, legal investigations, and observing for medications of manhandle. They may likewise be associated with examine, for instance in deciding the pharmacokinetic and toxicokinetic properties of substances or the viability of new treatment regimens.

9.3 DEVELOPING AN ANALYTICAL TOXICOLOGY SERVICE

When arranging the advancement of a scientific toxicology benefit there is various contemplations. These incorporate the example of harming and, accordingly, the particular substances for which investigations will be required, the current framework, the accessibility of progressing specialized help, save parts and reagents from providers, the accessibility of a unit of prepared staff and the ability to prepare new staff and give proceeding with proficient advancement.

9.4 ANALYTICAL TECHNIQUES SUITABLE FOR LOW-RESOURCE SETTINGS

IPCS has built up manual depicting straightforward scientific procedures for the distinguishing proof of more than 100 substances usually engaged with intense harming episodes. These methods don't require refined hardware or costly reagents, or even a consistent supply of power, and can be completed in the essential labs that are accessible to most clinics and wellbeing offices.

9.5 TOXIC RESPONSES OF BLOOD

Hematotoxicology is the investigation of antagonistic impacts of medications, non-remedial chemicals and different specialists in our condition on blood and blood-shaping tissues. Hematotoxicity might be viewed as essential, where at least one blood segments are straightforwardly influenced and optional, where the lethal impact is an outcome of other tissue damage or foundational aggravations. Essential harmfulness is viewed as among the more typical genuine impacts of xenobiotics, especially sedates. Auxiliary poisonous quality is exceedingly normal, because of the affinity of platelets to mirror an extensive variety of nearby and fundamental impacts of toxicants on different tissues. The reason for blood is to transport oxygen from the lungs around the body and carbon dioxide from the cells to the lungs for exhalation, to expel squanders, for example, lactic corrosive from muscle cells, to convey supplements, for example, amino acids and glucose to cells around the body, to keep up homeostasis and to clump wounds and battle disease. The indispensable capacities that platelets perform, together with the weakness of this exceedingly proliferative tissue to inebriation, makes the hematopoietic framework interesting as an objective organ. The crucial capacities that platelets perform, together with the defenselessness of this exceptionally proliferative tissue to inebriation, make the hematopoietic framework extraordinary as an objective organ. It positions with liver and kidney as among the most vital contemplations in the hazard appraisal of individual patient populaces presented to potential toxicants in nature, work environment and prescription bureau. The conveyance of oxygen to tissues all through the body, support of vascular honesty, and arrangement of the numerous affector and effector insusceptible capacities fundamental for have safeguard require a monstrous proliferative and regenerative limit. With intestinal mucosa and gonads, this makes hematopoietic tissue an especially delicate focus for cytoreductive or antimitotic

operators, for example, those used to treat disease, contamination, and safe intervened issue. This tissue is likewise vulnerable to optional impacts of lethal specialists that influence the supply of supplements, for example, press; the leeway of poisons and metabolites, for example, urea; or the creation of key development factors, for example, erythropoietin and granulocyte state invigorating component (G-CSF). The outcomes of immediate or aberrant harm to platelets and their forerunners are possibly perilous. They incorporate hypoxia, discharge and contamination. The generation of platelets, or hematopoiesis, is an exceedingly managed arrangement of occasions by which platelet antecedents multiply and separate to meet the tenacious needs of oxygen transport, have safeguard and repair, hemostasis, and other key capacities .with a specific end goal to comprehend hematotoxicology one should first comprehend the parts of the blood, their motivation, and their site of creation. There are three fundamental sorts of platelets, the erythrocyte (the red platelet) the leukocyte (the white platelet) and the thrombocyte (the platelet). Each of the three courses in the veins in a liquid part, the plasma or serum.

1.The Erythrocyte: The essential capacity of the erythrocyte in the body is the vehicle of oxygen and carbon dioxide. Red platelets are profoundly specific for their motivation. They are biconcave and have no core. RBCs course in the blood for around 120 days before they are expelled to the spleen where the iron is reused to frame new heme particles. Their film as in different cells is comprised of phospholipid bilayer, proteins and sugars. The typical red platelet check is more often than not in the vicinity of 4 and 6 million cells for each m3. Erythrocytes take up a gigantic measure of the aggregate blood volume. The fundamental capacity of the erythrocyte is as a transporter it go about as a bearer and store for any exogenous chemicals which enter the body. This makes erythrocytes exceptionally delicate to the nearness of such chemicals in the body. Poisons can change the structure, generation, work and even the survival of an erythrocyte bringing about an adjustment in the volume of erythrocytes in the blood which, considering their capacity in conveying oxygen to cells and expulsion of waste is greatly risky. Xenobiotics influence the creation, capacity and survival of erythrocytes. These impacts are most oftentimes shows an adjustment in the circling red cell mass, generally bringing about a decline. The operators that influence the oxygen partiality of hemoglobin prompt an expansion in the red cell mass (erythrocytosis). The adjustment in red platelet volume prompts an expansion in red platelet volume or a decline in red platelet volume. A decline in red platelet volume is generally normal. This is called pallor. Sickliness can be caused in two distinctive routes, first by pulverization of red

platelets and also by the diminished generation of red platelets. Poisons because an expansion in the quantity of circling erythrocytes called erythrocytosis, basically a thickening of the blood. Erythrocyte creation is a constant procedure that is subject to visit cell division and a high rate of hemoglobin amalgamation. Blend of hemoglobin is subject to facilitated generation of globin chains and heme moieties. Variations from the norm that prompt diminished hemoglobin union are generally normal (e.g., press inadequacy) and are frequently connected with a decline in the MCV and hypochromasia i.e. expanded focal paleness of RBCs. Xenobiotics influence globin-chain amalgamation and adjust the piece of hemoglobin inside erythrocytes. This is shown by hydroxyurea, which has been found to build the union of g globin chains. A medication that adds to blood misfortune, for example, non-steroidal calming operators, with their expanded danger of gastrointestinal ulceration and dying, potentiate the danger of creating iron lack sickliness. Imperfections in the amalgamation of porphyrin ring of heme prompt sideroblastic pallor, with trademark collection of iron in bone marrow erythroblasts. The amassed press hastens inside mitochondria, causing the intracellular damage. Various xenobiotics meddle with at least one of the means in erythroblast heme union and result in sideroblastic weakness. Numerous hostile to proliferative operators utilized as a part of the treatment of threat restrain hematopoiesis, including erythropoiesis. Various xenobiotics, especially mixes containing sweet-smelling amines, are fit for actuating oxidative damage in erythrocytes.

2. Haemoglobin makes up ~95% of the aggregate volume of a red platelet and is in charge of oxygen transportation. It is a tetramer, comprising of four polypeptide chains, 2 alpha globulin and 2 beta globulin. A heme gather comprises of an iron particle and a porphyrin ring, a natural compound. As the structure there are four heme gatherings; each of which can tie an oxygen particle. Hemoglobin partiality for oxygen is inconceivably high and subsequently, a red platelet which enters the lung turns out to be very soaked. This gives different territories where the nearness of poison in the blood meddle, for example, diminishing iron, an imperfection in the amalgamation of the porphyrin ring structure and modifications to the polypeptides of the tetramer. Methaemoglobin is shaped by the oxidation of oxyhaemoglobin. Methaemoglobin can't transport oxygen and is shaped by a substantial number of chemicals. The respiratory capacity of hemoglobin is weakened by the official of different ligands to the coupling site for instance carbon monoxide, a typical type of harming.

3.The Leukocyte: The leukocyte is a gathering of platelets (the white platelets) which have numerous parts. These incorporate the basophils, neutrophils and eosinophils which are all things considered known as the granulocytes, the monocytes and the lymphocytes. Granulocytes and monocytes are phagocytic in nature, immersing and devastating remote bodies. Both are nucleated and not at all like red platelets, fit for leaving the circulatory framework. As an extensive piece of the resistant reaction it enables the phagocytes to enter different tissues and demolish assaulting pathogens or cells which have experienced corruption or apoptosis. Aggravation and the safe reaction cause a tremendous increment in the quantity of coursing WBCs, including juvenile WBCs which are discharged trying to help the safe reaction. Change in morphology of neutrophils is a pointer of the nearness of a harmful specialist. Lethal impacts on granulocytes are wide and shifted. As in erythrocytes, high expansion in leukocytes makes granulocytes especially delicate to poisons in the blood. Operators like cisplatin are dangerous to both resting and isolating cells. The capacity of granulocytes is influenced by substances like liquor and glucocorticoids, which debilitate phagocytosis. Superoxide generation, which is obligatory for chemotaxis and microbial demise, turn out to be low in sedative abusers and in patients utilizing paraenteral heroin. In agranulocytosis an enormous drop in the quantity of flowing neutrophils happens. Xenobiotic instigates sudden agranulocytosis, cocurrent with presentation to the medication, and will endure as long as the medication which was the reason is in the framework. The patient more often than not recuperate once the medication has left the framework gave danger of disease is checked, however a poison which impacts non submitted foundational microorganisms can prompt aggregate marrow disappointment, for example, in a plastic frailty.

4. Carbon Monoxide: Environmental factors, for example, poisons inspire a lethality reaction in the body. Carbon monoxide is an unscented and bland harmful gas that is delivered as a result of the burning of hydrocarbons. Introduction to elevated amounts of carbon monoxide for a specific timeframe evoke lethal reactions in the blood; carbon monoxide presentation occur through the accompanying means: poor establishment of warming gadgets, blocked smokestacks and vapor from auto debilitates. The course of passage of carbon monoxide is through the lungs by inward breath, it is circulated around the body by aloof dispersion. Inactive. For an atom to be capable of latently diffuse it must be lipophillic, there must be a focus slope along the layer and the particle must be ionized. Carbon monoxide causes intense harmful impacts in the blood; the CO particle ties to hemoglobin to shape a carboxy-hemoglobin complex, this keeps oxygen atoms from having

the capacity to tie to the hemoglobin and subsequently oxygen can't be transported to fundamental tissues, for example, the mind and the heart. Absence of circulation of oxygen makes cells wind up noticeably anoxic, this at last prompt conditions, for example, cardiovascular tissue disappointment, mind harm and eventually cell passing. The level of danger caused by CO harming is reliant on term of introduction and the centralization of CO measured in parts per million.

9.6 ORGAN FUNCTION TESTS

Gathering of individual test used to assess organ usefulness or an aggregate term for an assortment of individual tests/methodology that assess organ work Useful to identify the nearness of illness, checking reaction to treatment and assessing visualization. Organ work tests are useful instruments in assessing the wellbeing status of a person. The tests are finished utilizing an organ framework approach i.e. renal, hepatic, gastric, and pancreatic and so on. There are a few factors, for example, race, eat less, age, sex, menstrual cycle and level of physical action, issues with accumulation and treatment of the example, non-physician endorsed drugs, doctor prescribed medications and various non-disease related components which influence tests. The organ work tests are named either obtrusive or non-intrusive tests.

9.6.1 INVASIVE TESTS

These tests require entrance of the skin or inclusion of instruments or gadgets into a body hole. The level of hazard with intrusive tests fluctuates from moderately minor dangers, for example, the torment, dying, and wounding related with venipuncture.

Examples of intrusive tests are gathering of blood (venipuncture), addition of a focal venous catheter, accumulation of cerebrospinal liquid and so on.

9.6.2 NON-INVASIVE TESTS

In these tests, the skin isn't infiltrated or instruments are not embedded into body openings. They posture little hazard to the patient.

Examples of non-obtrusive tests are chest radiograph, examination of unexpectedly voided pee, stool mysterious blood investigation and so on.

9.6.3 RENAL FUNCTION TEST

The fundamental capacity of the kidney is discharge of water dissolvable waste items from our body. The kidney has different filtration, discharge and secretary capacities. Unsettling of any of this capacity would bring about either diminished discharge of waste items or consequently their aggregation in the body or loss of some key supplement from the body. By the level of these excretory items and supplements in the pee and in blood we can make a precise figuring to translate the productivity of the kidney. The practical unit of the kidney is known as a nephron. It comprises of two fundamental parts, the glomerulus and the tubular framework. The glomerulus is made out of a bowman's case and a tuft of cracked veins epitomized by the Bowman's case. The basic role of the glomerulus is filtrations. The flawed vessels channel into the glomerulus all the water, electrolytes, little proteins, supplements, for example, sugar and so on and excretory items, for example, urea and so on. The filtration is reliant on the size and charge of the particles. The Tubular framework is in charge of re assimilation of the majority of the water, electrolytes, and supplements and additionally discharges of the rest of the supplements by methods for emission into the tubules. These tubules are in charge of the convergence of pee. The segments of the Kidney work test can be extensively isolated into two classes i.e. tests that measure the glomerular capacity and tests that measure the tubular capacity.

The tests that are a piece of the kidney/renal function test board are:

a) **Urine examination:** This examination comprises of a physical examination where the shading, smell, amount, specific gravity and so on of the pee is noted. Minute examination of pee is done to preclude any discharge cells, RBC throws, Crystals. Likewise a subjective examination is done as to give signs to the nature and area of the sore in the renal framework.
b) **Serum Urea:** Urea is the final result of protein catabolism. The urea is created from the amino gathering of the amino acids and is delivered in the liver by methods for the Urea cycle. Urea experiences filtrations at the glomerulus and also emission and reabsorption at the tubular level. The ascent in the level of serum urea is by and large observed as a marker of renal brokenness particularly glomerular brokenness. Urea level just ascents when the glomerular capacity is lessened underneath half. The ordinary serum urea level is between 20-45 mg/dl. The level is influenced by eat less carbs and in addition non

kidney related disarranges. Different reasons for protein catabolism, for example, hyper metabolic conditions, starvation and so on causes expanded blood urea levels; the level of urea additionally diminishes in the event of hepatic damage. Urea is measured in indicative lab by UV active strategy utilizing á ketoglutarate as a NH3+ acceptor in nearness of chemical glutamate dehydrogenase. It is likewise measured calorimetrically by Berthelot's end point technique and read in obvious range utilizing a calorimeter.

c) **Blood urea nitrogen (BUN):** The Serum urea level is communicated as blood urea nitrogen, BUN is computed from the serum urea level. The sub-atomic weight of urea is 60 and it contains two nitrogen particles of consolidated nuclear weight of 28. The commitment of nitrogen to the aggregate weight of urea in serum is 28/60 that is equivalent to 0.47. Henceforth the serum urea levels are changed over to BUN by increasing it by 0.47. An ascent in blood nitrogen level is known as azotemia.

d) **Calcium:** This test measures the measure of Calcium in blood, not the calcium in bones. The body needs it to assemble and settle bones and teeth enable nerves to work, make muscles constriction, enable blood to cluster, and help the heart to work. The Calcium test is for issues with the parathyroid organs or kidneys, certain sorts of growths and bone issues, aggravation of the pancreas (pancreatitis), and kidney stones. Typical Results: 8.5 to 10.2 mg/dl.

e) **Phosphorus:** Phosphorus is a mineral that makes up 1% of a man's aggregate body weight. The body needs phosphorus to manufacture and repair bones and teeth, enable nerves to capacity, and influence muscles to contract. The Kidneys help control the measure of phosphate in the blood. Additional phosphate is sifted by the kidneys and goes through of the body in the pee. It assumes an essential part in the body's usage of sugars and fats and in the combination of protein for the development, support, and repair of cells and tissues. Large amounts of phosphorus in blood just happen in individuals with extreme kidney infection or serious brokenness of their calcium direction. Unnecessarily abnormal amounts of phosphorus in the blood are uncommon.

f) **Protein:** Protein in pee is recognizably expanded in renal infection of any etiology, aside from obstacle, and is in this way an extremely touchy, general screening test for renal illness. The degree of proteinuria gives valuable data as more noteworthy the level of proteinuria in the nephritic disorder (> 3 - 4 g/day). In renal ailment with the nephritic disorder, the urinary protein discharge rate is as a rule around 1 - 2 g/day. In tubulo-interstitial ailment, pee protein is for the most part under 1 g/day. Just in the nephritic

disorder the pee protein misfortune adequately extraordinary to bring about hypoproteinemia.

g) **Serum creatinine:** Creatine is a little tripeptide found in the muscles. It remains in its phosphorylated frame and discharges vitality for any burst of solid action. It is discharged from the muscles amid normal wear and tear and is changed over to creatinine (its interior anhydride). It isn't a poisonous waste. It is basically utilized as a marker of renal capacity. Creatinine is uninhibitedly separated at the glomerulus and a little degree emitted into the tubules. So any issue with gromerular filtrations significantly affects the discharge of creatinine bringing about a much substantialrise in serum creatinine level. Ordinary serum creatinine level is 0.6 to 1.5 mg/dl. Serum creatinine is a superior pointer of renal capacity and more particularly glomerular capacity than urea. For a specific individual the creatinine level is subject to the bulk and muscle wear and tear. There might be noteworthy distinction in creatinine level of people with tremendously contrasting bulk. Creatinine is measured calorimetrically by Jaffe's technique. Eg. A muscle head or competitor will have higher creatinine levels than an inactive work area specialist.

h) **Creatinine freedom:** Creatinine is separated in the glomerulus and its reabsorption at the tubular level is irrelevant. On account of this creatinine freedom is utilized to quantify Glomerular Filtration Rate (GFR). It is measured for a time of 24 hrs. For this pee is gathered for 24 hour time frame and blood test likewise gathered. The grouping of creatinine is measured both in the pee and additionally the serum test. The ordinary scope of creatinine leeway is: Male: 100 – 120 ml/min, Females: 95 – 105 ml/min

i) **Urea clearance:** Urea freedom is the measure of blood from which kidney clears urea in one moment. This is measured by measuring the centralization of urea in blood, convergence of urea in pee and measure of pee discharged over a one hour interim. Urea leeway is not as much as its glomerular filtration as a portion of the urea that is separated at the glomerulus is reabsorbed at the tubules. To gauge urea leeway first the patient is made to void pee and afterward he is made to drink two glasses of water. At that point the pee is gathered following a hour and a blood example is additionally gathered in the meantime. At that point the patient pee test is gathered after one more hour. The urea level in the two pee tests and the blood test is measured. The pee volume is figured as pee yield every moment. Most extreme urea freedom of a normal individual or body surface range of 1.73sq m is 75 ml/min and a standard urea leeway is 54 ml/min. A urea freedom underneath 60% of standard is viewed as hindered.

j) **Inulin leeway:** Inulin is a little polysaccharide of low atomic weight made up of fructose. To gauge glomerular filtrate the substance utilized ought to have the qualities like; It ought to be non dangerous, Should not be processed in the body, Should be totally separated at the glomerulus, neither should nor be discharged or reabsorbed at the tubules. GFR is the measure of blood that passes however and is separated through the glomerulus in a moment. To gauge Inulin freedom first Inulin is presented in the blood by methods for an ease back consistent mixture to keep up an enduring conc. of Inulin in the blood. This is finished by first mixing 30 ml of 10% inulin in 250 ml of ordinary saline implanted at a rate of 20 ml/min for wanted fixation. At that point 70 ml of 10% inulin in 500 ml saline is mixed at a rate of 4 ml/min to keep up the coveted focus. The patient is made a request to micturate 20 minutes after the second mixture and the pee is disposed of and the time is noted. After precisely a hour, another specimen of pee and blood is gathered. The volume of pee and the conc. of inulin is measured in both the serum and pee. Ordinary inulin freedom is 120 to 130 ml/minute for a normal individual with a body surface territory of 1.73 sq m. An underneath typical inulin leeway demonstrates a weakened glomerular capacity.

k) **Dilution test:** The weakening test is a measure of working of the tubules. If there should be an occurrence of liquid over-burden of our body the tubules reabsorb lesser measures of water bringing about discharge of weakened pee. For this test the patient is put on overnight quick and after that in the morning the patient is made to drink 1200 ml of water over an era of 30 minutes. At that point the pee tests are gathered each hour for 4 hours. The particular gravity of the examples is measured and atleast one of the specimens ought to have a particular gravity of 1.003 or less. In the event that none of the specimens have the particular gravity of 1.003 or less this is an indication of tubular brokenness.

l) **Concentration test:** if there should be an occurrence of water lack in the body the kidney can think pee and preserve water. This is finished by expanding the reabsoption of water from the glomerular filtrate at the tubular level. So to quantify the capacity of the kidney to preserve water and think pee is a measure of tubular capacity. For this test the patient isn't permitted to take any sustenance or water after the night feast. The initial three pee tests go in the morning are gathered and their particular gravity is measured. In an ordinary individual the particular gravity of one of the specimens ought to be over 1.025

or above. On the off chance that the particular gravity stays beneath 1.025 then it is an indication of tubular brokenness.

m) Electrolyte levels: The reason for the kidney isn't simply water adjust and discharge yet in addition to keep up the electrolyte adjust of our body. Kidneys effectively reabsorb or discharge electrolytes to keep up the electrolyte adjust of the body. Inferable from their little size all electrolytes are separated at the glomerulus. After filtration a large portion of the electrolytes are retained back at the tubular level yet any issue at the tubular level will bring about non ingestion and over the top loss of electrolytes in pee.

9.6.4 HEPATIC FUNCTION TEST

Liver capacity tests are a gathering of tests done to evaluate the useful limit of the liver and any phone harm to the liver cells. It is done to evaluate utilitarian capacities like engineered capacity i.e. by measuring the different plasma proteins, for example, egg whites and prothrombin that are orchestrated by the liver; additionally lipids which are likewise combined in the liver and its secretory/excretory capacities by measuring the serum billirubin level. The regular tests that frame some portion of the liver capacity test profile are Serum Bilirubin both conjugated and unconjugated, add up to serum proteins and egg whites globulin proportion, liver catalysts transaminases AST (SGOT), ALT (SGPT) others are ALP, GGT, LDH and Prothrombin time.

Serum Bilirubin: Bilirubin is one of the final results of haem digestion and is gotten from the haem part of the hemoglobin atom. It is a yellow hued shade. Liver assumes a critical part in the digestion of bilirubin. After the breakdown of haem segment of the hemoglobin atom 'unconjugated bilirubin' is insoluble in water. It is exchanged from the site of RBC and haem breakdown, for example, the spleen to the liver for 'conjugation' bound to egg whites. At the liver it is conjugated with glucoronic corrosive with the assistance of catalyst glucuronyl transferase. This conjugation influences bilirubin to water dissolvable and this conjugated bilirubin is discharged into the bile. The typical scope of bilirubin is; add up to Bilirubin 0.2 to 1 mg/dl, Unconjugated Bilirubin 0.1 to 0.6 mg/dl, and Conjugated bilirubin 0.1 to 0.4 mg/dl. An ascent of bilirubin level to that of 2 mg/dl brings about the side effects of jaundice which is set apart by testimony of bilirubin in the different mucous films. Jaundice is partitioned into three sorts relying upon its etiology:

a) **Pre hepatic jaundice:** For this situation the reason for jaundice is at the level of bilirubin preparing before it achieves the liver. Most normal reason is over creation of bilirubin because of hemolytic issue. For this situation the ascent in the level of unconjugated bilirubin is more than conjugated bilirubin subsequently there is an ascent altogether and circuitous bilirubin.
b) **Hepatic jaundice:** This is caused by cell brokenness of the liver thus is additionally called hepatocellular jaundice. It is caused by the powerlessness of the liver cells to process and discharge the bilirubin in the framework. It is found in hepatitis, cirrhosis of liver and so forth. In this jaundice there is ascend altogether, immediate and additionally roundabout bilirubin levels.
c) **Post hepatic jaundice:** This is otherwise called obstructive jaundice as it is caused by obstacle to the outpouring of bile coming about is reabsoption of conjugated bilirubin and it showing up in the serum. It is caused via carcinoma of the mouth of bother bladder, stone in the bile conduit and so forth. In this kind of jaundice an ascent altogether and in addition coordinate (conjugated) bilirubin is seen.

The basic technique for measuring serum bilirubin level is the Diazo strategy utilizing Diazotized sufanilic corrosive to change over bilirubin into an azobilirubin the shading power of which is measured colorimetrically at a wavelength between 555nm (550 to 580 nm).

Serum Albumin and Albumin Globulin Ratio: Serum egg whites is an imperative serum protein key for keeping up the plasmaoncotic weight and additionally goes about as a bearer for different natural substances and medications. Serum egg whites are solely blended by liver and consequently the level of serum egg whites gives us a supply of the manufactured capacity of the liver. The reason for fall of serum egg whites is protein unhealthiness. The typical aggregate protein level is 5 to 8.5 gm/dl. The aggregate serum egg whites level is 3.5 to 5 gm/dl. The aggregate plasma globulin level is ascertained by subtracting the plasma egg whites from the aggregate protein level and is ordinarily in the scope of 2 to 2.5 gm/dl. The ordinary range for albumin:globulin proportion is 1.2 to 1.5. Yet, with hepatic brokenness this proportion retreats towards 1 as the manufactured capacity of liver is traded off. The inversion of the proportion i.e. in the event that the esteem subsides underneath 1, it is an unfavorable sign and may check an infective/provocative pathology set apart by ascend in serum globulin level and fall in serum egg whites levels. To quantify serum egg whites the Bromocresol green strategy is utilized. Egg whites within the sight of bromocresol green at a

somewhat acidic pH give a yellow green to blue geen shading. The power of this shading is subject to the convergence of egg whites in the specimen. This force is perused at a wavelength of 630 nm. To gauge the aggregate protein substance of the specimen the biuret technique it utilized. In this technique the cupric particles of copper (II) sulfate, display in the biuret reagent, frame a violet shaded complex with the proteins in a somewhat antacid medium. The force of the shading framed is measured at a wavelength of 540 nm (530 to 550 nm).

Prothrombin Time: Prothrombin is a coagulating factor (thickening variable II) and it frames a vital piece of both the characteristic and extraneous pathway. Its dynamic frame is Thrombin (additionally coagulating factor IIa). It is a serine peptidase which changes over fibrinogen to fibrin. Prothrombin is orchestrated in the liver. Furthermore, subsequently prothrombin action in plasma is utilized to quantify the engineered capacity of liver. Prothrombin time is measured by taking human plasma from blood that has been gathered in tube containing citrate as an anticoagulant. The plasma is placed in a computerized machine which adds an abundance of calcium to switch the anticoagulant impacts of citrates and measures the time taken for fibrinogen to be changed over to fibrin subsequently measures the movement of thrombin in the plasma. The prothrombin time contrasts in understanding to the expository technique utilized. An International affectability record (ISI) esteem is set apart on the unit; this demonstrates the measure of tissue calculate exhibit the pack against a universally acknowledged standard. The ISI esteem is by and large 1 to 2.

A proportion of 0.8 to 1.2 is viewed as typical for patients not on warfarin. For people on warfarin for any confusion an INR of 2.0 to 3.0 is the objective.

Liver Enzymes: Liver catalysts alongside bilirubin are the most normally measured parameter measured in the liver capacity test. These compounds are hepatic in source and they are spilled into the serum with the annihilation of hepatic cells. Liver chemicals are measured to get a thought of the cell affront on the liver and are expanded in a wide assortment of conditions, for example, viral hepatitis, dangerous hepatitis, and cirrhosis of liver and so on. The ordinarily measured chemicals Transaminases: AST (SGOT), ALT (SGPT, Transpeptidases: GGT and Phosphatase: ALP.

a) **Transaminases:** They are a gathering of catalysts that exchange the amino gathering from an amino corrosive to α keto corrosive changing over the α keto corrosive into

an amino corrosive while changing over the amino corrosive into a keto corrosive. The transaminases that are measured in the liver capacity test are ALT and AST. Alanine transaminase (ALT) catalyzes the accompanying response:

Alanine + α keto Glutarate −A−LT−→ Pyruvate + Glutamate

Aspartate transaminase (AST) catalyzes the accompanying response:

Aspartate + α keto Glutarate −A−ST−→ Oxaloacetate + Glutamate

The ordinary level of ALT in serum is 7 to 40 IU/L. The typical level of AST in serum is 8 to 40 IU/L. An expansion in AST or ALT levels indications at damage to the liver parenchyma tissue. ALT is a more particular marker of hepatic damage than AST as AST height is additionally observed in cardiovascular tissue damage, haemolysis and muscle tissue. To quantify the level of transaminases the response catalyzed by them is coupled to a response in which NADH is spent bringing about change in the photometric force when perused in the UV run at 340 nm. It is an UV dynamic strategy.

For ALT (SGPT)

Alanine + α Keto glutarate −A−LT−→ Pyruvate + Glutamate

Pyruvate + NADH + H+ LDH (Lactate dehydrogenase) ————→ Lactate + NAD+

For AST (SGOT)

Aspartate + α Keto glutarate −A−ST−→ Oxaloacetate + Glutamate

Oxaloacetate + NADH + H+ MDH (Malate dehydrogenase) ————→ Malate + NAD+

b) Alkaline Phosphatase: It is a hydrolase that expels phosphates from a wide range of atoms, for example, proteins, nucleotides and so on. It is found in cells coating the billiary framework subsequently an ascent in it level is characteristic of harm to the billiary tree because of cholestasis. It is because of stone hindering the substantial pipes or intrahepatic impediment, aggravation of the biliary channels. Alkailine phosphatase is likewise found in placenta and bones. Subsequently the level is additionally expanded in developing youngsters in whom bones experience redesigning and in Paget's ailment in grown-ups. Typical level of antacid phosphatase is between 45 to 115 IU/L. The strategy for measuring the level of antacid phosphatase is an active technique utilizing p-nitrophenylphosphate as substrate for the compound and measuring rate of arrangement of the shaded substrate (p-

nitrophenol) shaped from the response. This estimation of the shading power is done colorimetrically at a wavelength of 405 nm.

c) **Gamma glutamyl transpeptidase:** It is another catalyst particular to the biliary tree and a more particular marker of cholestasis and harm to the biliary tree. It is likewise an exceptionally particular marker and is brought up in even moment and subclinical harm to the biliary tree. It's typical range is in the middle of 0 to 42 IU/l.

9.6.5THYROID FUNCTION TESTS

Thyroid capacity tests are utilized to build up the level of thyroid capacity (e.g., hyperthyroid, hypothyroid, euthyroid) and the reaction to suppressant or substitution treatment. Thyroid capacity is surveyed by assessing the serum groupings of the free hormones thyroxine (T4) and triiodothyronine (T3) and by various aberrant strategies.

Free Thyroxine Index: The free thyroxine record (FT4I) is the result of the deliberate T4 and the triiodothyronine take-up (T3U). It considers the outright hormone level and the coupling limit of thyroid-restricting globulin. The FT4I is diminished in hypothyroidism and expanded in hyperthyroidism.

Thyroid-Stimulating Hormone (Thyrotropin): Serum TSH, or thyrotropin, levels are utilized to separate between thyroid hypothyroidism and pituitary hypothyroidism. The TSH level is raised in thyroidal hypothyroidism and uniquely diminished in pituitary hypothyroidism.

Thyroid Uptake of Radioiodine: Radioactive iodine (123I or 131I) is controlled orally, and the radioactivity over the thyroid organ is checked at different interims. The typical radioactive iodine take-up (RAIU) is around 10% to 35%.

Thyrotropin-Releasing Hormone: Thyrotropin-discharging hormone (TRH) fortifies the pituitary to discharge TSH. Infusion of manufactured TRH regularly causes an expansion in TSH in around 30 minutes.

Triiodothyronine Uptake: The triiodothyronine take-up (T3U) test is an in vitro test that by implication evaluates the measure of thyroid-restricting globulin in the serum.

9.6.6PANCREATIC FUNCTION TESTS

Amylase: Amylase is discharged by the pancreas, inside, parotids, and gynecologic framework. Despite the fact that not particular for pancreatitis, serum amylase is less demanding to gauge than is lipase and is utilized as a typical screening and observing parameter for intense pancreatitis. Notwithstanding, in constant pancreatitis the pancreas might be "wore out" and unfit to discharge amylase.

Peptide: C peptide is an idle peptide chain discharged from beta cells in equimolar sums with insulin and found in the serum in around a 5:1 to 15:1 proportion with insulin. C peptide is now and again used to survey pancreatic capacity.

Glucose: Serum glucose focuses are utilized to survey pancreatic capacity and the reaction to insulin substitution treatment.

Fasting Serum Glucose: The serum test is gotten following 10 to 14 hours of fasting. The fasting serum glucose is normally gotten before breakfast after an overnight quick.

Glucose Tolerance Test: The glucose resilience test (GTT) is utilized to analyze diabetes mellitus and gestational diabetes. Patients quick for 10 to 16 hours before the test and are then gave around 75 g of glucose. Serial blood tests are acquired, and the serum glucose fixation is resolved. Regularly, the serum blood glucose is under 200 mg/dl at 30, 60, and a hour and a half and under 140 mg/dl at 2 hours.

Irregular Serum Glucose: The arbitrary serum glucose test can be gotten whenever without fasting.

Glycosylated Hemoglobin: Glycosylated hemoglobin is shaped when hemoglobin is irreversibly glycosylated after presentation to high glucose levels. Glycosylated hemoglobin surveys long haul control of insulin treatment and separates factitious hyperglycemia from diabetes.

Insulin: Fasting serum insulin is here and there gotten amid the evaluation of pancreatic capacity.

Lipase: Lipase is a particular marker for intense pancreatic ailment. Increments in serum lipase parallel increments in serum amylase. Be that as it may, in interminable pancreatitis the pancreas might be "wore out" and unfit to discharge lipase.

9.6.7 CARDIOVASCULAR FUNCTION TEST

Cardiac Enzymes: The example and time course of the presence of compounds in the blood after cardiovascular muscle cell harm are utilized to analyze myocardial localized necrosis (MI).

Creatine Kinase (CK; creatine phosphokinase): It is found in skeletal muscle; heart muscle; and the cerebrum, bladder, stomach, and colon. Isoenzyme portions distinguish the kind of tissue harmed. CK-BB (CK1) is found in the mind, bladder, stomach, and colon; CK-MB (CK2) is found in heart tissue; and CK-MM (CK3) is found in skeletal muscle. CK-MB is recognized in the blood inside 3 to 5 hours after a myocardial dead tissue; levels crest in around 10 to 20 hours and standardize inside around 3 days.

Lactic Dehydrogenase (LDH): It is found in an assortment of body tissues. Isoenzyme parts are utilized to distinguish the sort of tissue damage.LDH1 and LDH2are found in the heart, cerebrum, and erythrocytes. LDH2 regularly represents the most noteworthy level of aggregate serum LDH. After a myocardial localized necrosis (MI) the ascent in LDH1 focus surpasses the ascent in LDH2 fixation. LDH3 is found in the cerebrum and kidneys. LDH4 is found in the liver, skeletal muscle, and kidneys. LDH5 is found in the liver, skeletal muscle, and ileum. LDH increments inside around 12 hours after a myocardial dead tissue, top in the vicinity of 24 and 48 hours, and standardizes by about day 10.

Cholesterol: Cholesterol is isolated into lipoproteins by protein electrophoresis. Low-thickness lipoprotein (LDL) is emphatically associated with coronary course illness. High thickness lipoprotein (HDL) is conversely connected with coronary course ailment.

Responsive protein: C-receptive protein is a biologic marker of fundamental irritation. Increment in C-receptive protein focus expands danger of myocardial localized necrosis, stroke and fringe blood vessel infection.

Myoglobin: Myoglobin is a little protein found in heart and skeletal muscle. The nearness of myoglobin in the pee or plasma is a moderately delicate marker of cell harm.

Triglycerides: Triglycerides are found in low-thickness lipoproteins (VLDLs) and chylomicrons.

Troponins: These are mind boggling proteins (troponin I, C, and T) that intercede the actin and myosin cooperation in muscle. Troponin I and T are particular to cardiovascular muscle and are utilized to recognize heart muscle damage. Troponin I and T fixations increment inside a couple of hours of heart muscle damage and stay lifted for 5 to 7 days.

Teratogenic: The synthetic species that reason birth absconds are called teratogens. These species harm embryonic or fetal cells which result in birth surrenders. Be that as it may, transformations in germ cells (egg or sperm cells) may cause birth abandons. The biochemical components incorporate compound restraint by xenobiotics ;(synthetic substances that are unfamiliar to living frameworks), hardship of fundamental substrates for embryo, for example, vitality and vitamins and adjustment of the penetrability of placental layer.

9.7 REPRODUCTIVE TEST

9.7.1 HYSTEROSALPINGOGRAM

Hysterosalpingogram (HSG) is a x-beam that analyzes within your uterus and fallopian tubes. This test can enable your specialist to check whether your fallopian tubes are blocked. This test can be exceptionally useful, since blocked tubes are one of the more typical reasons for barrenness. Here is the manner by which the hysterosalpingogram procedure works:

1. Your specialist infuses a color through the vagina and cervix into the uterine cavity.
2. Dye fills the fallopian tubes on the off chance that they are open, yet may not enter the tubes on the off chance that they are blocked.
3. If the color spills out into your stomach cavity, your specialist will realize that your fallopian tubes are open.

A hysterosalpingogram can likewise enable your specialist to find any uterine variations from the norm, polyps, fibroids, and scar tissue. This methodology may make mellow direct

cramping.

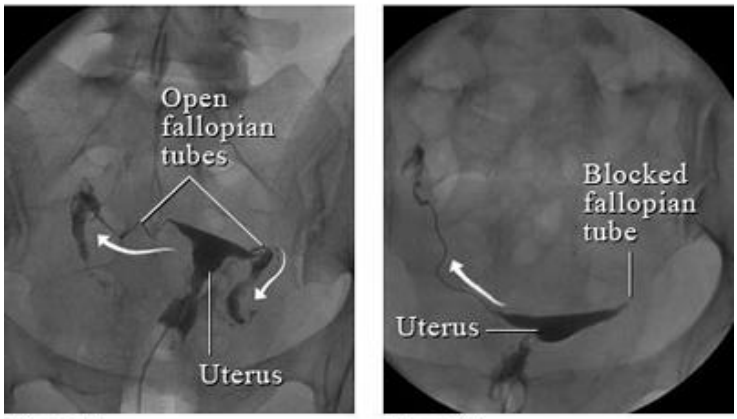


Figure 1 Figure 2

Fig 10.1 Hysterosalpingogram (HSG)

9.7.2 LAPAROSCOPY

In the event that pelvic variations from the norm are associated causes with barrenness, your specialist may suggest laparoscopy, a negligibly obtrusive surgery. Here is the means by which it works:

1. Your specialist will embed a laparoscope (a little fiber optic telescope) into your mid-region.
2. Through little entry points (0.5 cm), your specialist can investigate your fallopian tubes, ovaries, and uterus.
3. In this technique, your specialist has the capacity to view and treat pelvic structure irregularities. He or she can likewise treat blockages, scar tissue, and other harm.

Recuperation from laparoscopy is fairly fast, and conveys a generally safe for creating attachments (sinewy scar tissue that can cause fruitfulness issues).

9.7.3 HYSTEROSCOPY

Hysteroscopy distinguishes and treat uterine conditions. Here is the means by which it works:

1. Your specialist embeds a thin hysteroscope (a fiber optic telescope) through the cervix and into the uterus.
2. During this system, your specialist can see your uterine depression and analyze fibroids, polyps, scar tissue, and different issues.

3. Hysteroscopy might be performed in the workplace while you are conscious, or as an operation under general anesthesia.

9.7.4 SALINE HYSTEROGRAM

A saline hysterogram (SHG) is otherwise called a saline implantation sonogram (SIS), water ultrasound, or sono-hysterogram. Here is the way it works:

1. An SHG happens amid a transvaginal ultrasound.
2. Your specialist infuses saline into your cervix and uterus to watch the dividers of your uterus.
3. This method enables your specialist to recognize any developments in the uterine dividers, similar to fibroids or polyps.

9.7.5 ENDOMETRIAL BIOPSY

An endometrial biopsy can enable your specialist to distinguish uterine, menstrual, and hormonal scatters that might be reasons for barrenness. On the off chance that you experience the system, here is what's in store:

1. Your specialist will give you a pregnancy test with a specific end goal to secure a conceivable pregnancy.
2. The biopsy will occur a few days before your period begins.
3. A little specimen of tissue will be taken from the endometrium (internal uterine covering) for perception.

A few ladies encounter torment and cramping amid and after the method.

9.7.6 CERVICAL MUCUS TEST AND POSTCOITAL TEST

Now and again, issues with cervical bodily fluid (CM) can hinder pregnancy. The cervical bodily fluid test and postcoital test (PCT) can offer your specialist some data on the quality and consistency of your CM. It can likewise offer data on how your accomplice's sperm cooperates with your CM. A decent time to assess your CM is around the season of the LH surge, just before ovulation happens.

Shouldn't something be said about protection?

Some protection designs cover ripeness tests and treatment, and a few issues can be effectively treated. Treatment may not be as costly as you think.

Richness testing can bring solace

Going through richness testing may feel overpowering and obtrusive. Locate a regenerative endocrinologist you trust and express your worries.

Keep in mind, your specialist had numerous claims to fame to look over and the one they picked was regenerative endocrinology. They have devoted their professions to helping men and ladies – like you – understand their fantasy of turning into a parent and are delicate to the passionate toll fruitfulness testing and treatment can take.

Transvaginal ultrasound. A specialist puts a ultrasound "wand" into the vagina and conveys it near the pelvic organs. Utilizing sound waves, he'll have the capacity to see pictures of the ovaries and uterus to check for issues there.

9.8 CARCINOGENIC TEST

"The term cancer-causing agent indicates a synthetic substance or a blend of compound substances which incite tumor or increment its frequency" by **UNECE, 2004**. A substitute definition is that cancer-causing substances are ones that "prompt tumors (amiable or threatening), increment their rate or danger, or abbreviate the season of tumor event when they are breathed in, infused, dermally connected, or ingested" (**Maurici, et al., 2005**).

Cancer-causing agents are arranged by their method of activity as genotoxic or nongenotoxic cancer-causing agents. Genotoxic cancer-causing agents start carcinogenesis by coordinate communication with DNA, bringing about DNA harm or chromosomal distortions that can be recognized by genotoxicity tests (OECD, 2006). Nongenotoxic cancer-causing agents will be operators that don't straightforwardly cooperate with DNA and are accepted to upgrade tumor improvement by influencing quality articulation, flag transduction, as well as cell multiplication. In creature thinks about, most powerful mutagens are likewise observed to be cancer-causing (Maurici, et al., 2005, p. 177). Substances that actuate tumors in creatures are considered as assumed or suspected human cancer-causing agents until the point when persuading proof in actuality is displayed (UNECE, 2004, p. 167).

9.8.1THE ANIMAL TEST(S)

The customary test for cancer-causing nature is the long haul rat cancer-causing nature bioassay depicted in Organization for Economic Cooperation and Development (OECD) Test Guideline (TG) 451. The target of this test is "to watch test creatures for a noteworthy bit of their life expectancy for the advancement of neoplastic sores amid or after presentation to different measurements of a test substance by a fitting course of organization." The examination is normally directed utilizing two species – rats and mice of both genders. The creatures are dosed by oral, dermal, or inward breath exposures, in view of the normal kind of human introduction. Dosing commonly keeps going around two years. Certain creature wellbeing highlights are observed all through the examination, yet the key appraisal lives in the full obsessive investigation of the creature tissues and organs when the investigation is ended.

Two endpoints in creature bioassays, cancer-causing nature and perpetual harmfulness, can be consolidated to diminish creature use, as depicted in OECD TG 453.

The International Life Sciences Institute (ILSI) Health and Environmental Science Institute's (HESI) Alternatives to Carcinogenicity Testing Technical Committee facilitated an extensive scale inquire about program to describe various transgenic rat models proposed for use in human tumor hazard evaluation by **Robinson and MacDonald, 2001**. None of these models were viewed as adequate as independent examines. Most could recognize genotoxic aggravates that a genotoxicity test battery would as of now identify, however better discovery of nongenotoxic cancer-causing agents is as yet required by **Goodman, 2001**.

Controllers at a 2003 ILSI-HESI workshop on the utilization of transgenic creatures for cancer-causing nature testing presumed that these measures ought to be incorporated with customary test strategies (ILSI-HESI, 2003). The controllers considered the p53+/ - and Tg.RasH2 models valuable in giving information to administrative purposes and the Tg.AC display helpful in assessing dermal items.

9.8.2 REGULATORY REQUIREMENTS & TEST GUIDELINES

The UN Globally Harmonized System (GHS) characterizes cancer-causing agents under two classifications in view of the quality of the confirmation: Category 1 chemicals are known or assumed human cancer-causing agents (Category 1A if in light of human information and 2A

if in view of creature information); Category 2 chemicals are suspected human cancer-causing agents (UNECE, 2004, p. 167). As per GHS direction, concoction prompted tumorigenesis includes hereditary changes; in this manner, chemicals that are mutagenic in warm blooded creatures may warrant being named cancer-causing agents.

The GHS portrays other "critical elements" to be thought about in cancer-causing agent danger characterization, for example, the area and number of tumors, tumor sort and attributes, reactions in both genders and additionally numerous species, importance of the method of activity to people, and the sky is the limit from there. The OECD's direction on these variables is given in the 2001 Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (ENV/JM/MONO(2001)6), and in the 2005 Proposal for Guidance on How to Consider Important Factors in Classification of Carcinogenicity (ENV/JM/HCL(2005)2/REV). The 2005 OECD direction talks about different systems for surveying the "vital factors" and expresses that "the heaviness of confirmation investigation called for in GHS is an integrative approach which considers imperative factors in deciding cancer-causing potential alongside the quality of proof examination."

OECD TGs 451, 452, and 453 give data to leading cancer-causing nature and endless lethality thinks about. The OECD Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies (ENV/JM/MONO (2002)19) "gives wide direction on ways to deal with risk appraisal and on a portion of the issues and traps that may emerge amid an evaluation."

The US Environmental Protection Agency's (EPA) reconsidered its Guidelines for Carcinogen Risk Assessment (EPA/630/P-03/001B) in 2005. The changed rules utilize five descriptors (Carcinogenic to Humans, Likely to be Carcinogenic To Humans, Suggestive Evidence of Carcinogenic Potential, Inadequate Information to Assess Carcinogenic Potential, Not Likely to Be Carcinogenic to Humans) that are trailed by a weight of proof account to depict the cancer-causing capability of a substance. The EPA gives extra data on its Web page Evaluating Pesticides for Carcinogenic Potential.

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and medication administrative specialists give direction on testing to the cancer-causing capability of new medications. Long haul danger

concentrates, for example, cancer-causing nature testing are generally led simultaneously with clinical trials. Jena, et al. (2005) give a decent diagram of cancer-causing nature testing for sedate improvement.

The International Agency for Research on Cancer (IARC), some portion of the World Health Organization (WHO), gives Monographs on the Evaluation of Carcinogenic Risks to Humans and has assessed the cancer-causing danger of more than 900 substances. "The IARC Monographs are perceived as a definitive wellspring of data" and might be utilized by national and universal experts in making hazard evaluations.

9.8.3 NON-ANIMAL ALTERNATIVE METHODS

Non-creature techniques incorporate cell-based tests and computational forecast models. Mutagenicity and genotoxicity measures can be utilized to demonstrate conceivable cancer-causing substances, and the two in vitro strategies depicted underneath (cell change and hole intersection intercellular correspondence) can be utilized to recognize conceivable cancer-causing agents, including nongenotoxic cancer-causing agents. Contrasted with the in vivo cancer-causing nature measures, in vitro strategies are fundamentally speedier and more affordable, yet current techniques are not viewed as adequate to fill in as full creature substitutions as of now.

Cell change examines (CTA) depend on distinguishing phenotypic changes instigated by chemicals in mammalian cell societies. The most generally utilized of these tests are the Syrian hamster fetus (SHE) examine, the low-pH SHE test, the Balb/c 3T3 measure, and the C3H10T1/2 test (Maurici, et al., 2005). The SHE test is accepted to recognize early strides of carcinogenesis, and the Balb/c and C3H10 measures later cancer-causing changes (OECD, 2006). These examines decide the cytotoxicity of test substances by measuring impacts on morphology, state framing capacity, as well as development rate (Combes, et al., 1999). "Collected confirmation unequivocally bolsters the presumption that phone and sub-atomic procedures engaged with cell change in vitro are like those of in vivo carcinogenesis" (Combes, et al., 1999; OECD, 2006). An ECVAM prevalidation venture on SHE and Balb/3T3 examines is under way. The hole intersection intercellular correspondence (GJIC) technique depends on the interruption of the intercellular trade of low-atomic weight particles through the hole intersections of nearby cells; this disturbance can bring about strange cell development and conduct (Maurici, et al., 2005a). The examine has all the earmarks of being

a decent possibility for screening for nongenotoxic cancer-causing agents and tumor promotors, however despite everything it should be institutionalized and approved.

Quantitative and subjective structure-action relationship models (QSARs and SARs) and master frameworks have been created to anticipate cancer-causing nature. A few late productions have looked into models, for example, TOPKAT, CASE, and DEREK, utilized by administrative experts (Cronin, et al., 2003; OECD, 2007). All in all, the calculation of cancer-causing nature is unpredictable, and prescient ability has been restricted. The US FDA financed the advancement of MultiCASE in view of information from administrative entries, and it was accounted for to have enhanced predictivity (Cronin, et al., 2003).

Mutagenicity/genotoxicity examines are the most ordinarily utilized as a part of vitro test frameworks to anticipate cancer-causing nature. Mutagenicity alludes to the enlistment of transmissible changes in the structure of the hereditary material of cells or life forms (Maurici, et al., 2005b). Changes may include a solitary quality or a gathering of qualities. Genotoxicity is a more extensive term that alludes to changes to the structure or number of qualities by means of substance communication with DNA as well as nonDNA targets, for example, the shaft device and topoisomerase chemicals (Maurici, et al., 2005b). The term genotoxicity is by and large utilized unless a particular test is being talked about. Being used for more than 30 years, genotoxicity examines are utilized in a level testing approach that begins with Tier I in vitro tests, trailed by Tier II in vivo genotoxicity tests to decide the natural importance of chemicals that are sure in the in vitro tests. Regular genotoxicity testing batteries incorporate examines that measure transformations and also basic and numerical chromosome abnormalities (as looked into in Maurici, et al., 2005b).

Eight in vitro genotoxicity test techniques, four of which are usually utilized, have been received at the EU level with OECD rules (see table beneath). These four in vitro measures incorporate two mutagenicity test techniques in view of bacterial cells (the bacterial switch change test [Ames test], OECD TG 471; and the Escehrichia coli turn around transformation examine, OECD TG 472), and also two strategies in light of mammalian cells (the in vitro mammalian chromosome variation test, OECD TG 473; and the in vitro mammalian cell quality transformation test, OECD TG 476). Furthermore, the European Center for the Validation of Alternative Methods (ECVAM) approved the in vitro micronucleus test for genotoxicity testing in 2006 as a contrasting option to the in vitro chromosome deviation test (ESAC proclamation: 17 November 2006; modified OECD TG 487, in arrangement).

Various other in vitro genotoxicity tests, incorporating the in vitro Comet examine, are being produced yet are not yet approved.

Table 1. **OECD TGs** for in vitro genotoxicity and mutagenicity testing

TG 471	Bacterial Reverse Mutation Test (Ames Test)
TG 472	Genetic Toxicology: Escherichia coli, Reverse Assay
TG 473	In Vitro Mammalian Chromosome Aberration Test
TG 476	In Vitro Mammalian Cell Gene Mutation Test
TG 479	Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells
TG 480	Genetic Toxicology: Saccharomyces cerevisiae, Gene Mutation Assay
TG 481	Genetic Toxicology: Saccharomyces cerevisiae, Mitotic Recombination Assay
TG 482	Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells in vitro
TG 487	In vitro Mammalian Cell Micronucleus Test

A current examination of the execution of the most well-known in vitro genotoxicity tests for expectation of cancer-causing nature has been distributed (Kirkland, et al., 2005, p. 200). In this evaluation, a battery of three in vitro genotoxicity assays– the Ames test, the mouse lymphoma test (MLA), and the in vitro micronucleus (MN) or chromosomal changes (CA) test– separated between rat cancer-causing agents and noncarcinogens when each of the three tests were sure or every one of the three were negative (Kirkland, et al., 2005). The affectability of the information was high, yet the specificity of the mammalian measures was poor. Truth be told, 75-90% of rat noncarcinogens were sure in at least one of the tests, bringing about a high number of false positive outcomes. Along these lines, it isn't conceivable as of now to depend on current in vitro genotoxicity tests alone, without the Tier II in vivo genotoxicity tests.

9.8.4VALIDATION AND ACCEPTANCE OF NON-ANIMAL ALTERNATIVE METHODS

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and ECVAM has not formally approved any option techniques for cancer-causing nature testing right now.

ECVAM is leading a prevalidation/approval contemplate on three cell change examines: SHE cells at pH 6.7; SHE cells at pH 7.0; and Balb/c 3T3 cell line. An OECD TG is being drafted at the same time. The prevalidation think about stage was finished in 2010, and the Validation Management Team reasoned that "institutionalized conventions are presently accessible that ought to be the reason for sometime later. The SHE pH 6.7, and the SHE pH 7.0 conventions and the examines framework themselves are transferable amongst research facilities, and are reproducible inside and between-labs. For the Balb/c 3T3 strategy, a few illuminations and adjustments to the convention were expected to acquire reproducible outcomes. In general, three techniques have appeared to be profitable to identify rat cancer-causing agents." The outcomes from the prevalidation ponders are at present experiencing peer audit with the ESAC.

Because of the different phases of carcinogenesis, the long in vivo day and age required, the various instruments, and the requirement for metabolic transformation of a few substances, existing cell-based measures can be utilized just in a layered testing plan or test battery as an incomplete swap for the creature bioassays (Maurici, et al., 2005a). An ECVAM board couldn't give an expected date to the aggregate trade of creature testing for cancer-causing nature at the EU level (Maurici, et al., 2005a). An ECVAM board reasoned that aggregate swap of creature testing for genotoxicity isn't doable inside the following 12 years (Maurici et al., 2005b). The accompanying graph speaks to the course of events for approval of non-creature options for cancer-causing nature testing proposed by the board in 2005.

9.9 SUMMARY

The scientific toxicologist might be required to recognize, distinguish, and by and large measure a wide assortment of mixes in tests from any piece of the body or in related materials, for example, deposits in syringes or in soil. This book gives standards and pragmatic data on the examination of medications and toxins in organic examples, especially clinical and scientific examples.

Subsequent to giving some foundation data the book covers parts of test gathering, transport, stockpiling and transfer, and test arrangement. Explanatory procedures - shading tests and spectrophotometry, chromatography and electro¬phoresis, mass spectrometry, and immunoassay – are canvassed top to bottom, and a section is dedicated to the examination of follow components and dangerous metals. General parts of technique usage/approval and research center operation are itemized, similar to the part of the toxicology lab in approving and observing the execution of purpose of care testing (POCT) gadgets. The book closes with surveys of Toxic reaction of blood, organ work tests, teratogenic, conceptive test, cancer-causing test and general parts of the understanding of systematic toxicology comes about.

- A unmistakably composed, commonsense, incorporated way to deal with the nuts and bolts of systematic toxicology.
- Focuses on logical, measurable and pharmacokinetic standards instead of point by point applications.
- Assumes just fundamental information of diagnostic science.
- An going with site gives extra material and connections to related locales.

9.10 GLOSSARY

Absorption

The process of taking in. For a person or an animal, absorption is the process of a substance getting into the body through the eyes, skin, stomach, intestines, or lungs.

Acute

Occurring over a short time [compare with chronic].

Acute exposure

Contact with a substance that occurs once or for only a short time (up to 14 days) [compare with intermediate duration exposure and chronic exposure].

Additive effect

A biologic response to exposure to multiple substances that equals the sum of responses of all the individual substances added together [compare with antagonistic effect and synergistic effect].

Adverse health effect

A change in body functions or cell structure that might lead to disease or health problems

Aerobic

Requiring oxygen [compare with anaerobic].

Ambient

Surrounding (for example, ambient air).

Anaerobic

requiring the absence of oxygen [compare with aerobic].

Analyte

A substance measured in the laboratory. A chemical for which a sample (such as water, air, or blood) is tested in a laboratory. For example, if the analyte is mercury, the laboratory test will determine the amount of mercury in the sample.

Analytic epidemiologic study

A study that evaluates the association between exposure to hazardous substances and disease by testing scientific hypotheses.

Antagonistic effect

A biologic response to exposure to multiple substances that is **less** than would be expected if the known effects of the individual substances were added together [compare with additive effect and synergistic effect].

Background level

An average or expected amount of a substance or radioactive material in a specific environment, or typical amounts of substances that occur naturally in an environment.

Biodegradation

Decomposition or breakdown of a substance through the action of microorganisms (such as bacteria or fungi) or other natural physical processes (such as sunlight).

Biologic indicators of exposure study

A study that uses (a) biomedical testing or (b) the measurement of a substance [an analyte], its metabolite, or another marker of exposure in human body fluids or tissues to confirm human exposure to a hazardous substance [also see exposure investigation].

Biologic monitoring

Measuring hazardous substances in biologic materials (such as blood, hair, urine, or breath) to determine whether exposure has occurred. A blood test for lead is an example of biologic monitoring.

Biologic uptake

The transfer of substances from the environment to plants, animals, and humans.

Biomedical testing

Testing of persons to find out whether a change in a body function might have occurred because of exposure to a hazardous substance.

Biota

Plants and animals in an environment. Some of these plants and animals might be sources of food, clothing, or medicines for people.

Body burden

The total amount of a substance in the body. Some substances build up in the body because they are stored in fat or bone or because they leave the body very slowly.

Cancer

Any one of a group of diseases that occur when cells in the body become abnormal and grow or multiply out of control.

Cancer risk

A theoretical risk for getting cancer if exposed to a substance every day for 70 years (a lifetime exposure). The true risk might be lower.

Carcinogen

A substance that causes cancer.

Case study

A medical or epidemiologic evaluation of one person or a small group of people to gather information about specific health conditions and past exposures.

Case-control study

A study that compares exposures of people who have a disease or condition (cases) with people who do not have the disease or condition (controls). Exposures that are more common among the cases may be considered as possible risk factors for the disease.

CAS registry number

A unique number assigned to a substance or mixture by the American Chemical Society Abstracts Service .

Central nervous system

The part of the nervous system that consists of the brain and the spinal cord.

Chronic

Occurring over a long time.

Chronic exposure

Contact with a substance that occurs over a long time (more than 1 year)

Cluster investigation

A review of an unusual number, real or perceived, of health events (for example, reports of cancer) grouped together in time and location. Cluster investigations are designed to confirm case reports; determine whether they represent an unusual disease occurrence; and, if possible, explore possible causes and contributing environmental factors.

Community Assistance Panel (CAP)

A group of people from a community and from health and environmental agencies who work with ATSDR to resolve issues and problems related to hazardous substances in the community. CAP members work with ATSDR to gather and review community health concerns, provide information on how people might have been or might now be exposed to hazardous substances, and inform ATSDR on ways to involve the community in its activities.

Comparison value (CV)

Calculated concentration of a substance in air, water, food, or soil that is unlikely to cause harmful (adverse) health effects in exposed people. The CV is used as a screening level during the public health assessment process. Substances found in amounts greater than their CVs might be selected for further evaluation in the public health assessment process.

Completed exposure pathway Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA)

CERCLA, also known as Superfund, is the federal law that concerns the removal or cleanup of hazardous substances in the environment and at hazardous waste sites. ATSDR, which was created by CERCLA, is responsible for assessing health issues and supporting public health activities related to hazardous waste sites or other environmental releases of hazardous substances.

Concentration

The amount of a substance present in a certain amount of soil, water, air, food, blood, hair, urine, breath, or any other media.

Contaminant

A substance that is either present in an environment where it does not belong or is present at levels that might cause harmful (adverse) health effects.

Volatile organic compounds (VOCs)

Organic compounds that evaporate readily into the air. VOCs include substances such as benzene, toluene, methylene chloride, and methyl chloroform.

9.11 SELF ASSESSMENT QUESTIONS AND POSSIBLE ANSWERS

Multiple choice questions

1. Which of the following characterizes the use of vasopressors in management of a potential organ donor?
    a. Their use precludes procurement of the kidneys
    b. Only agents devoid of inotropic activity should be used
    c. Norepinephrine is the agent of choice
    d. The choice of agents must be individualized based on physiologic parameters
2. Which of the following electrolyte abnormalities results from the effects of brain death on the posterior pituitary gland?
    a. Hyponatremia b. Hypernatremia
    c. Hyperkalemia d. Hypokalemia

e. Hypocalcemia

3. Hormonal replacement therapy for brain dead donors includes administration of all of the following, EXCEPT:

   a. Growth hormone b. Vasopressin
   c. Thyroxine d. Corticosteroids

4. Which of the following immunosuppressants most commonly affects spermatogenesis?

   a. Sirolimus b. Tacrolimus
   c. Cyclosporine d. Azathioprine

5. The minimum allowable age for live kidney donors is:

   a. 14 years b. 16 years
   c. 18 years d. 21 years

Fill in the blanks:

1. Ethyl alcohol is considered to be a_____

   a. depressant b. hallucinogen
   c. stimulant d. narcotic

2. Marijuana is considered to be a_____

   a. hallucinogen b. nacrotic
   c. depressant d. stimulant

3. The direct allogeneic antigen presentation involves the recognition of peptides through intact MHC molecules displayed on _____ APCs while indirect allogeneic antigen presentation involves the recognition of _____ through self - MHC displayed on _____ APCs.

   a. Donor; host MHC; host b. Host; host MHC; donor
   c. Donor; donor MHC; host d. Host; donor MHC; donor

4. All of the following are features of cytomegalovirus syndrome, EXCEPT_____

   a. Fatigue b. Fever

c. Leukocytosis d. Thrombocytopenia

e. Myalgias

5. Transplanted _____ is mostly frequently affected by BK polyoma virus?

a. Kidney b. Heart

c. Lung d. Liver

Short questions:

1. Write short not on analytical taxonomy.
2. What are the toxic responses of blood?
3. Give short note on teratogenic?
4. What tests are used for fertility/infertility, give short description?
5. Write short notes on reproductive and carcinogenic test.

Long questions:

1. Define analytical taxonomy and its applications?
2. What are the toxic responses of blood?
3. Describe organ function tests.
4. What cause teratogenic, its cause and remedies?
5. Which tests are used to check reproductive and carcinogenic toxicity?.

9.12 REFERENCES

1. Carcinogenic Potency Database (CPDB): Easily accessed results of 6,153 experiments on 1,485 chemicals from 1,426 papers and 429 NCI/NTP (National Cancer Institute/National Toxicology program) Technical Reports.
2. Chemical Carcinogenesis Research Information System (CCRIS): Carcinogenicity and mutagenicity test results for more than 8,000 chemicals.
3. Combes, R., Balls, M., Curren, R., et al. (1999). Cell transformation assays as predictors of human carcinogenicity. ECVAM Workshop Report 39. Altern. Lab. Anim. 27, 745-767.
4. Cronin, M.T.D., Jaworska, J.S., Walker, J.D., et al. (2003). Use of QSARs in International Decision-Making Frameworks to Predict Health Effects of Chemical Substances. Environ. Health Perspect. 111, 1391-1401. Available here.

5. ECVAM. (2002). Genotoxicity and carcinogenicity. Altern. Lab. Anim. 30, Suppl. 1, 83-93.
6. Goodman, J.I. (2001). A perspective on current and future uses of alternative models for carcinogenicity testing. Toxicol. Pathol. 29, Suppl., 173-176.
7. Hendrix, M.J., Seftor, E.A., Seftor, R.E., et al. (2007). Reprogramming metastatic tumour cells with embryonic microenvironments. Nat. Rev. Cancer. 7, 246-255.
8. Junghans, T.B., Sevin, I.F., Ionin, B. & Seifried, H. (2004). Cancer information resources: Digital and online sources. Toxicol. 198, 177-193.
9. Maurici, D., Aardema, M., Corvi, R., et al. (2005a). Carcinogenicity. Altern. Lab. Anim. 33, Suppl. 1, 177-182.
10. United Nations Economic Commission for Europe (UNECE). (2004). Globally Harmonized System of classification and labeling of chemicals (GHS). Part 3, Health and environmental hazards. Chapter 3.6, Carcinogenicity. Available here.

BLOCK- IV

BIOTECHNOLOGY

UNIT: 10 RECOMBINANT DNA TECHNOLOGY

CONTENTS

10.1 OBJECTIVES

After studying this Unit, students will know about:

- The gene cloning and steps involved in it.
- Restriction enzymes, ligases, linkers and adaptors.
- Selection of recombinants, hybridisation techniques, gene probes, DNA fingerprinting, molecular markers used in genome analysis, genome library and blotting techniques used in recombinant DNA technology.

10.2 INTRODUCTION

Recombinant DNA Technology is a genetic engineering technology of joining together distinct DNA molecules to produce new genetic combinations as "recombinant DNA" (rDNA). In recombinant DNA technology, molecules of DNA from two different species are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry. A series of procedures are used to join together (recombine) DNA segments from two or more different molecules. Under certain conditions, a recombinant DNA molecule enters the cell and replicates autonomously inside the host and integrates with the host chromosome. Recombinant DNA technology was first developed in 1977 by Herbert Boyer, Paul Berg and Stanley N. Cohen, when they successfully expressed somatostatin in bacteria.

There are five steps involved in recombinant DNA technology:

The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.

Step1.Isolation of Genetic Material

The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.

Step2.Cutting the gene at the recognition sites

The restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome. These reactions are called 'restriction enzyme digestion'.

Step3.Amplifying the gene copies through Polymerase chain reaction (PCR)

It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using the restriction enzymes.

Step4.Ligation of DNA Molecules

This step of involves Ligation (joining) of the two pieces –a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase

Step5. Insertion of Recombinant DNA into Host

In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as Transformation. Once inserted into the host cell, the recombinant DNA gets multiplied and expressed in the form of the manufactured protein under optimal conditions.

10.3 GENE CLONING- THE BASIC STEPS

"DNA cloning, gene cloning or molecular cloning is a technique used to make multiple identical copies of a particular segment of DNA or gene." A clone is a cluster of individual entities or cells that are descended from one progenitor. Clones are genetically identical as the cell simply replicates producing identical daughter cells every time. Scientists are able to generate multiple copies of a single fragment of DNA, a gene which can be used to create identical copies constituting a DNA clone.

Cloning takes place through the insertion of DNA fragments into a tiny DNA molecule. This molecule is made to replicate within the living cell, for instance, a bacterium. The tiny replicating molecule is known as the carrier of the DNA vector. Yeast cells, viruses, Plasmids are the most commonly used vectors. Plasmids are the circular DNA molecules that are introduced from bacteria. They are not part of the main cellular genome. It carries genes, which provide the host cell with beneficial properties such as mating ability, drug resistance. They can be conveniently manipulated as they are small enough and they are capable of carrying extra DNA which is weaved into them.

Steps involved in gene cloning:

1. A gene segment is cloned and inserted into a circular DNA molecule i.e. plasmid vector to produce an r DNA.

2. The vector carries the gene segment into the host cell, mostly bacterium.

3. Inside the host cell, the vector multiplies, producing numerous identical copies of the DNA of the vector as well as the gene segment inserted.

4. When the host multiplies the r DNA passed to the daughter cells.

5. After many cell divisions, colonies of identical host cells are produced. Each cell contains one or more copies of r DNA molecules.

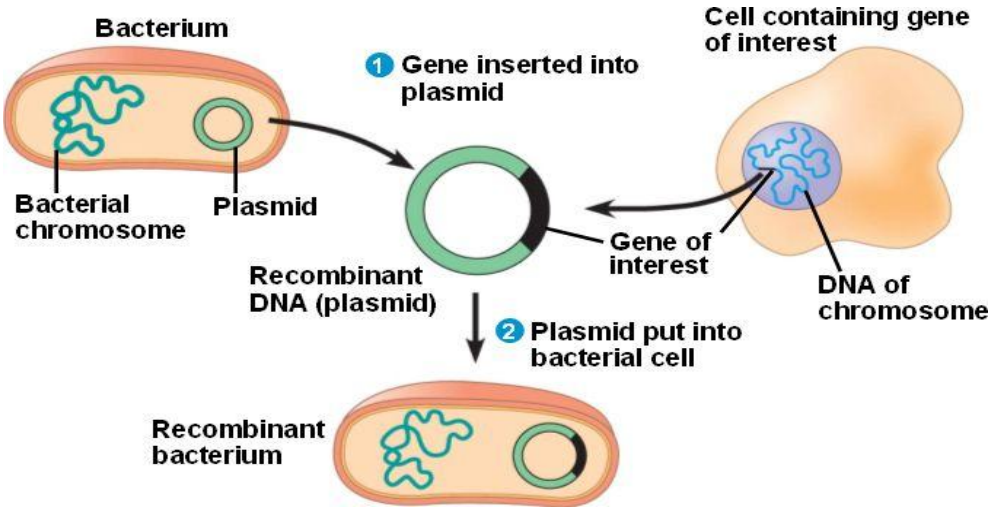


Figure 10.1: Gene cloning (Creative biolabs.com)

10.4 RESTRICTION ENZYMES LIGASE LINKERS AND ADAPTORS C DNA–TRANSFORMATION

Restriction enzymes are found in bacteria (and other prokaryotes). They recognize and bind to specific sequences of DNA, called **restriction sites**. A restriction enzyme is a protein that recognizes a specific, short nucleotide sequence and cuts the DNA *only* at that specific site, which is known as restriction site or target sequence.

Bacterium restriction-modification systems have two components- a restriction endonuclease and DNA methylase. Each restriction enzyme recognizes just one or a few restriction sites.

When it finds its target sequence, a restriction enzyme will make a double-stranded cut in the DNA molecule. Endonuclease is a nuclease enzyme which cleaves the nucleic acid chain within the DNA, rather than at the ends (exonuclease). The first restriction enzyme was isolated from *E. coli K-12*laboratory strains in 1968 by Meselson and Yuan.

A bacterium is immune to its own restriction enzymes, even if it has the target sequences ordinarily targeted by them. This is because the bacterial restriction sites are highly methylated, making them unrecognizable to the restriction enzyme. More than 400 restriction enzymes have been isolated from the bacteria that manufacture them. In live bacteria, restriction enzymes function to defend the cell against invading viral bacteriophages.

Restriction enzymes are named for the organism from which they were first isolated. For example: **1.***Eco*RI is isolated from the *E. coli* strain RY13.*Eco* refers to the genus and species (1st letter of genus; 1st two letters of specific epithet)R is the strain of *E. coli.* I (Roman numeral) indicate it was the first enzyme of that type isolated from *E. coli* RY13.
2. *BamHI* is isolated from *Bacillus amylolique faciens* strain H.

Linkers are short pieces of double stranded (ds) DNA, of known nucleotide sequence that is synthesized in the test tube i.e. synthetic oligonucleotides. These are blunt ends but contain a restriction site (BamHI). **DNA ligase** can attach linkers to the ends of larger blunt-ended DNA molecules. In a blunt end ligation, this particular reaction can be performed very efficiently because linkers can be made in very large amounts and added into the ligation mixture at a high concentration. More than one linker will attach to each end of the DNA molecule, producing the chain structure. However, digestion with BamHI cleaves the chains at the recognition sequences, producing a large number of cleaved linkers and the original DNA fragment, now carrying BamHI sticky ends. This modified fragment is ready for ligation into a cloning vector restricted with BamHI.

Adaptors, like linkers, are short synthetic oligonucleotides. But unlike linkers, an adaptor is synthesized so that it already has one sticky end. Using adaptors has one more problem. The sticky ends of individual adaptor molecules could base pair with each other to form dimers. This left the new DNA molecule still blunt-ended. The sticky ends could be recreated by digestion with a restriction endonuclease, but that would defeat the purpose of using adaptors in the first place. To solve this problem we remove the phosphate group from the 5′ position of sticky ends of the adaptors by the help of alkaline phosphatase enzyme. Due to this DNA

ligase is now unable to form a phosphodiester bridge between 5′-OH and 3′-OH ends. The result of this manipulation is that, although base pairing always occurs between the sticky ends of adaptor molecules, the association is never stabilized between them by ligation. Adaptors can, therefore, be ligated to a blunt-ended DNA molecule but not to themselves. After the adaptors have been attached, the abnormal 5′-OH terminus is converted to the natural 5′-P form by treatment with the enzyme polynucleotide kinase, producing a sticky-ended fragment that can be inserted into an appropriate vector.

Complementary DNA (cDNA)is a double stranded DNA synthesized in the laboratory from messenger RNA. cDNA is not genomic DNA, because the transcript of genomic RNA has been processed (i.e., it lacks promoters and introns). The enzyme reverse transcriptase is used to synthesize double-stranded cDNA that is a complimentary copy of the mRNA. Using an mRNA molecule as a template, reverse transcriptase synthesizes a single-stranded DNA molecule that can then be used as a template for double-stranded DNA synthesis. cDNA does not need to be cut in order to be cloned because it lacks introns. The addition of linker sequences to the end of this DNA, which contain the restriction site, followed by treatment with a restriction enzyme, produces a cDNA preparation with cohesive ends ready for insertion into a vector. A preparation of cDNA represents the genes that were actively being expressed in a cell, an organ, or a whole organism at the time of harvesting and is called a cDNAlibrary.

10.5 SELECTION OF RECOMBINANTS

The need to identify the cells that contain the desired insert at the appropriate and right orientation and isolate these from those not successfully transformed is of utmost importance to researchers. Modern cloning vectors include selectable markers that allow only cells in which the vector, but not necessarily the insert, has been transformed to grow. Additionally, the cloning vectors may contain color selection markers which provide blue/white screening (via α-factor complementation) on X-gal medium. The resulting colonies are required to confirm that cloning was successful. This may be accomplished by means of PCR, restriction fragment analysis and/or DNA sequencing.

1. Colony immunoassay

Colony hybridization was developed for screening recombinants where upon blotting onto nitrocellulose filters and hybridization with a highly radioactive probe, screening of many

thousands of colonies per plate for the presence of a DNA sequence carried by a plasmid and complementary to the probe was achieved.

An immunological approach to screen recombinant clones is possible if the gene of interest encodes a polypeptide for which specific antibodies can be prepared. In one approach, DNA complementary to mRNA is inserted in frame with the coding regions of genes present in *E. coli* plasmids. These results in "fused polypeptides" consisting of the N-terminal region of an *E. coli* polypeptide covalently linked to a sequence encoded by the cloned cDNA segment. The identification of cloned genes by colony immunoassays has not been common and one limitation of all previous colony immunoassays is that each fused polypeptide molecule must simultaneously bind to two different antibody molecules. Typically, the first antibody, immobilized on a solid support such as chemically activated paper, is used to entrap the fused polypeptide at the site of the lysed colony, and a second labelled antibody is then bound to the fused polypeptide and detected by autoradiography. A potential disadvantage of all immunological methods is that only one in six sequences inserted at random into the vector would have the orientation and frame consistent with translation into a recognizable fused polypeptide.

A simple immunoassay has been developed by Reggie and Comeron (1986) for isolation of a particular gene from a clone bank of recombinant plasmids. A clone bank of the DNA is constructed with a plasmid vector in *Escherichia coli* and filtered onto a hydrophobic grid membrane and grown up into individual colonies, and a replica was made onto nitrocellulose paper. The bacterial cells upon lysis are immobilized onto the nitrocellulose paper which is reacted with a rabbit antibody preparation made against the particular antigenic product to detect the recombinant clone which carries the corresponding gene. The bound antibodies can be detected easily by a colorimetric assay using goat anti-rabbit antibodies conjugated to horseradish peroxidase.

2. The blue-white screening

The blue white screening is one of the most common molecular techniques that allow detection of the successful ligation of genes of interest in vectors. The α-Complementation plasmids are among the most commonly used vectors for cloning and sequencing the DNA fragments, as they generally have a good multiple cloning site and an efficient blue-white screening system for identification of recombinants in presence of a histochemical dye, 5-

bromo-4-chloro-3-indolyl-β-d-galactoside (X-gal), and binding sites for commercially available primers for direct sequencing of cloned fragments.

The molecular mechanism for blue/white screening is based on a genetic engineering of the lac operon in the *E. coli* as a host cell combined with a subunit complementation achieved with the cloning vector. The lacZ product, a polypeptide of 1029 amino acids, gives rise to the functional enzyme after tetramerization and is easily detected by chromogenic substrates either in cell lysates or directly on fixed cells in situ.

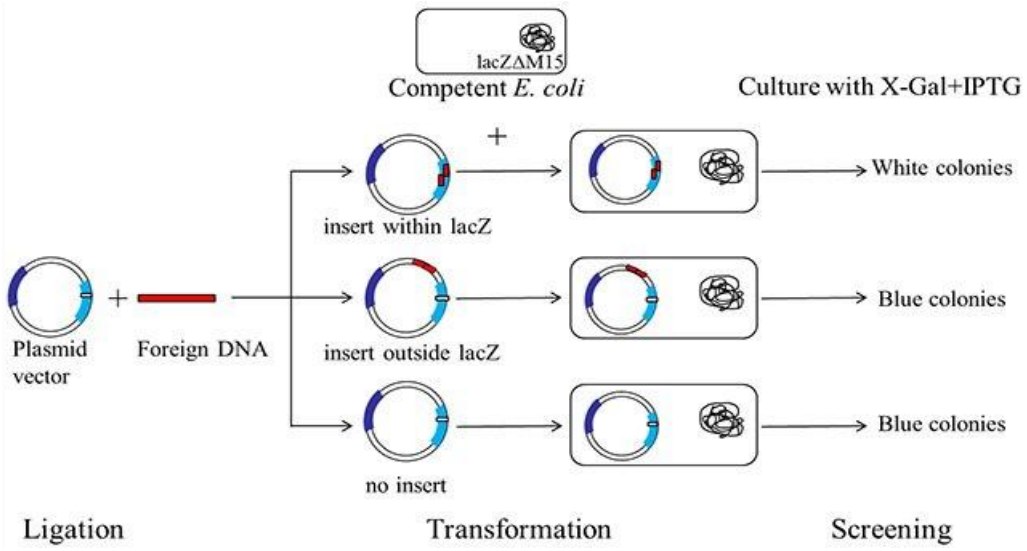


Figure 10.2: Selection of recombinants using blue white screening in Escherichia coli. (Source: proprofs)

The hydrolysis of colorless X-gal by the β-galactosidase causes the characteristic blue colour in the colonies indicating that the colonies contain vectors without insert. White colonies indicate insertion of foreign DNA and loss of the cell's ability to hydrolyse the marker.

Bacterial colonies in general, however, are white, and so bacterial colonies with no vector will also appear white. These are usually suppressed by the presence of an antibiotic in the growth medium. Blue white screening is thus a quick and easy technique that allows for the screening of successful cloning reactions through the colour of the bacterial colony.

3. Reporter gene based screening

Another method for screening and identification of recombinant clones is by using the green fluorescent protein (GFP) obtained from jellyfish *Aequoreavictoria*. It is a reporter molecule for monitoring gene expression, protein localization, protein-protein interaction etc. GFP has been expressed in bacteria, yeast, slime mold, plants, drosophila, zebrafish and in mammalian cells. The bacterial cloning vector, pGreenscript A, derived from the mutated Aequorea green

fluorescent protein (GFP-S65A) gene, when expressed in *E. coli* produced colonies that showed yellow color under daylight and strong green fluorescence under long-wave ultraviolet light. The vector is used to select for inserted foreign genes based on the loss of the yellow color/green fluorescence of E. coli cells caused by the insertional inactivation of GFP production.

4. Screening clones by positive selection

A variety of positive selection cloning vectors has been developed that allow growth of only those bacterial colonies that carry recombinant plasmids. Typically, these plasmids express a gene product that is lethal for certain bacterial hosts and insertion of any DNA fragment that insertionally inactivates the expression of the toxic gene product, results in growth of colonies.

Positive selection has been a powerful method of screening insert containing transformants. Here the toxic property of the molecule to the host cells is utilized for recombinant selection.

The DNA sequence coding for the toxic product is directly cloned under the promoter elements recognized by the host cells. Positive selection in these vectors is achieved by either inactivation or replacement of toxic genes by the target gene. In general former is much more convenient than the latter. The advantage of these systems is that no background colonies (non-recombinants) appear on vector alone plates since the relegated vectors carrying toxic intact genes are lethal to the host cells.

10.6 HYBRIDIZATION TECHNIQUES

DNA is usually found in the form of a double-stranded molecule. These two strands bind to one another in a complementary fashion by a process called hybridization. DNA, when replicated naturally, its new strand hybridizes to the old strand. In the laboratory we can take advantage of hybridization by generating nucleic acid probes which we can use to screen for the presence or absence of certain DNA molecules or RNA molecules in the cell.

Hybridization methods represent standard techniques in molecular biology. In general, they are used to detect particular sequences (target) within a complex mixture of DNA or RNA molecules. DNA or RNA are usually transferred and immobilized to nitrocellulose or, more commonly, to nylon membranes. Complementary single-stranded probes are labelled radioactively or non-radioactively. When hybridized to the filter, probes bind to their complementary target sequence via hydrogen bonds. Unhybridized probe is then washed away, and specifically-bound probe is detected by autoradiography or color reaction.

Applications of direct probe hybridization in diagnostic microbiology include identification of bacteria in blood culture bottles using fluorescence *in situ* hybridization (FISH). In diagnostic molecular pathology *in situ* hybridization is used to detect gene duplications and gene mutations (e.g., *in situ* hybridization for HER-2 amplification). Southern blotting, is an another hybridization-based method used in genetics assays to assess for large alleles, such as those that may be seen in triplet repeat disorders such as Fragile X syndrome or Huntington disease.

10.7 GENE PROBE- MOLECULAR FINGERPRINTING (DNA FINGERPRINTING)

Gene probe (DNA probe) is a single-stranded DNA or RNA fragment used in genetic engineering to search for a particular gene or other DNA sequence. The probe has a base sequence complementary to the target sequence and will thus attach to it by base pairing. Gene probes can be produced in several ways and fall broadly into three types: gene-specific probes, oligonucleotide probes and polymorphic probes.

Gene-specific probes are produced from specific mRNA by the enzyme reversetranscriptase, which synthesizes a complementary DNA copy (cDNA) from mRNA. If radioactive bases are added to the reaction mixture, the cDNA will be labelled and can thus be used as a hybridization probe to look for the complementary sequences.

Probes can be used in dot-blot hybridisation, where serial dilutions of DNA samples are held on DNA-binding membranes and the complementary radioactively labelled probes are hybridized *in vitro*, so that the amount of radioactive signal is proportional to the amount of target DNA present.

The cDNA can also be cloned by synthesizing a second DNA strand from cDNA using a bacterial DNA polymerase, which is incorporated into a plasmid and grown in bacterial cells. These **oligonucleotide probes** recognize short sequences of DNA that correspond to the sequence known to occur in the gene. With a probe of this length, a single mismatched base pair is sufficient to impair hybridization and can be used to detect changes to a single base (point mutations). Similarly, probes can be developed that recognize the various DNA polymorphisms within the non-coding sequences, for example RFLP and VNTR. DNA probes can also be used to identify abnormal genes or gene products at the molecular level

within cell cytoplasm or nucleus, by in situ hybridization. This technique utilizes labelled DNA or mRNA probes which hybridize to the expressed genes in the cell in a manner similar to that used for immunohistochemistry. In situ hybridization can thus establish whether the genomic material of interest is present in the DNA of the cell in vitro.

DNA fingerprinting:

DNA Fingerprinting is a molecular biology technique which was first developed in 1984 by British geneticist Alec Jeffreys. Sir Alec Jeffrey noticed that certain sequences of highly variable DNA (i.e., VNTRs), which do not contribute to the functions of genes, are repeated within genes are known as minisatellites. Jeffreys recognized that each individual has a unique pattern of minisatellites. Therefore, this technique can assist in the identification of individuals or samples by their respective DNA profiles. This was the main idea behind this marvelous invention.

More than 99.1% of the genome is same throughout the human population, the remaining 0.9% of human DNA shows variations between individuals. These variable DNA sequences, termed molecular markers, can be used to both differentiate and correlate individuals.

The procedure for creating a DNA fingerprint involve following steps:

1. Obtain a sample of cells, such as skin, hair, or blood cells, which contain DNA.
2. The DNA is extracted from the cells and purified.
3. The DNA was then cut at specific points along the strand with restriction endonuclease enzymes which are known as molecular scissors.
4. This enzyme produces fragments of varying lengths.
5. These fragments are then separated by gel electrophoresis technique.
6. The shorter the fragment, the more quickly it moved toward the positive pole (anode).
7. The sorted double-stranded DNA fragments were then subjected to a blotting technique in which they were split into single strands and transferred to a nylon sheet.
8. The fragments underwent autoradiography in which they were exposed to DNA probes (i.e., pieces of synthetic DNA) that were made radioactive and that bound to the minisatellites.

9. A piece of X-ray film was then exposed to the fragments, and a dark mark was produced at any point where a radioactive probe had become attached. The resultant pattern of marks could then be analyzed.

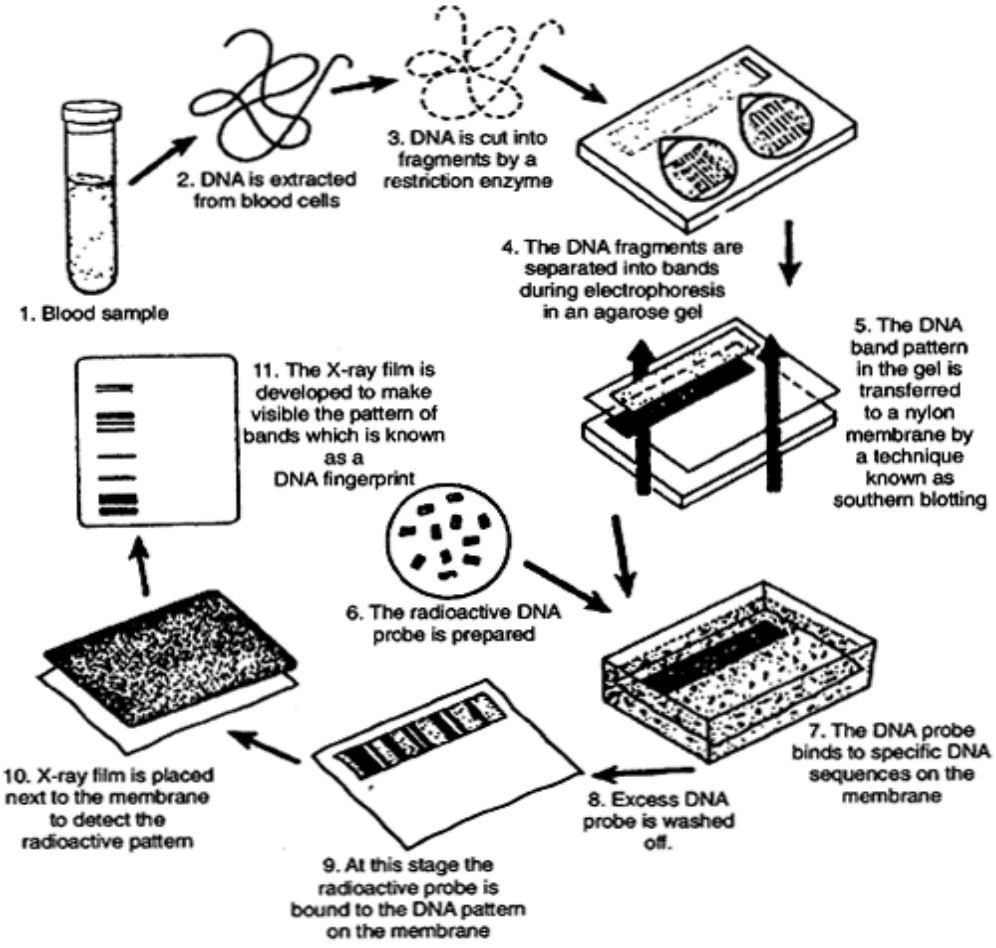


Figure 10.3: Steps involved in DNA fingerprinting (source: pinterest.com)

10.8 MOLECULAR MARKERS IN GENOME ANALYSIS (RFLP, RAPD AND AFLP)

A molecular marker is a molecule contained within a sample taken from an organism (biological markers) or other matter. DNA, for example, is a molecular marker containing information about genetic disorders and the evolutionary history of life.

Molecular markers help in detection of variations or polymorphisms that exist among individuals in the population for specific regions of DNA (e.g. RFLP, AFLP, SNP, etc.). Applications of Molecular markers in gene mapping are:

(1) A marker allows the direct identification of the gene of interest instead of the gene product, and consequently, it serves as a useful tool for screening somatic cell hybrids.

(2) Use in several DNA probes and easy-to-screen techniques, a marker also helps in the physical mapping of the genes using *in situ* hybridization.

(3) Molecular markers provide sufficient markers for construction of genetic maps using linkage analysis.

1. Restriction fragment length polymorphism: A Restriction fragment length polymorphism (or RFLP) is a variation in the DNA sequence of a genome that can be detected by breaking the DNA into pieces with restriction enzymes and analysing the size of the resulting fragments by gel electrophoresis. It is the sequence that makes DNA from different sources different, and RFLP analysis is a technique that can identify some differences in sequence (when they occur in a restriction site). Though DNA sequencing techniques can characterize DNA very thoroughly, RFLP analysis was developed first and was cheap enough to see wide application. Analysis of RFLP variation was an important tool in genome mapping, localization of genetic disease genes, determination of risk for a disease, genetic fingerprinting, and paternity testing.

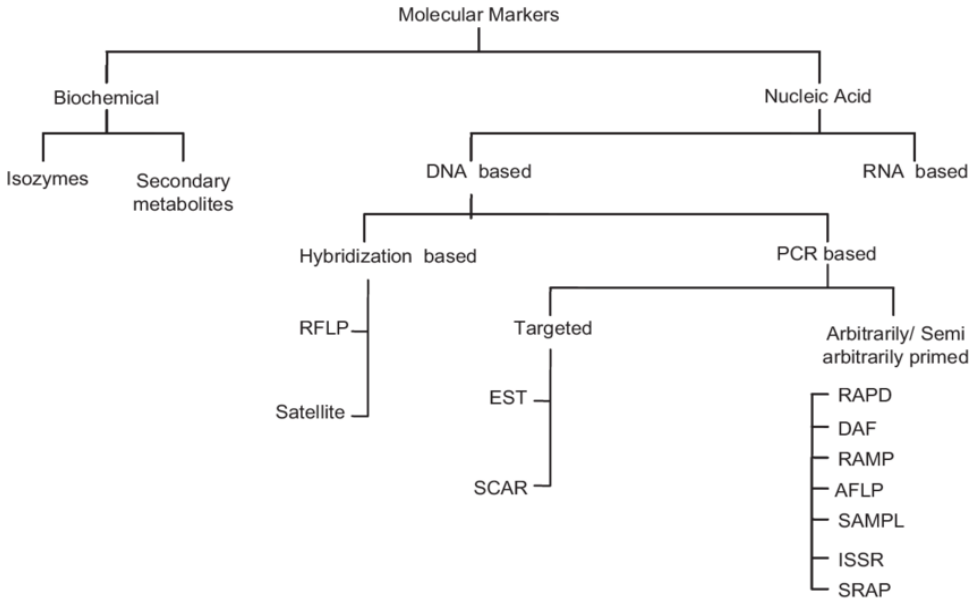
2. RAPD (Random Amplification of Polymorphic DNA)

It is a type of PCR reaction, but the segments of DNA that are amplified are random. The scientist performing RAPD creates several arbitrary, short primers (8-12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction.No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems that have not had the attention of the scientific community, or in a system in which relatively few DNA sequences are compared (it is not suitable for forming a DNA databank). Due to the fact that it relies on a large,intact DNA template sequence, it has some limitations in the use of degraded DNA samples. Its resolving power is much lower than targeted, species specific DNA comparison methods, such as short tandem repeats. In recent years, RAPD is used to characterize, and trace, thephylogeny of diverse plant and animal species.Random Amplified Polymorphic DNA (RAPD) markers are ten mer (10 nucleotide length) DNA fragments from PCR

amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way.

3. Amplified fragment length polymorphism: Amplified fragment length polymorphism PCR (or AFLPPCR or just AFLP) is a PCR-based tool used in genetics research, DNA fingerprinting, and in the practice of genetic engineering. AFLP uses restriction enzymes to cut genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments are then amplified using primers complementary to the adaptor and part of the restriction site fragments (as described in detail below). The amplified fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies. AFLP-PCR is a highly sensitive method for detecting polymorphisms in DNA. The technique was originally described by Vos and Zabeau in 1993.

In detail, the procedure of this technique is divided into three steps:
1. Digestion of total cellular DNA with one or more restriction enzymes and ligation of restriction half-site specific adaptors to all restriction fragments.
2. Selective amplification of some of these fragments with two PCR primers that have corresponding adaptor and restriction site specific sequences.
3. Electrophoretic separation of amplicons on a gel matrix, followed by visualisation of the band pattern. Flowchart: Dipicting categorisation of molecular markers



10.9 GENOMIC LIBRARY

A genomic library is a collection of overlapping segments of genomic DNA, cloned into a backbone vector, which statistically includes all regions of the genome of an organism. The resulting cloned DNA is then transformed into a suitable host cell line. Construction of a genomic library is an important initial step in many genetic studies or in the isolation and cloning of genes from an organism. Screening of genomic libraries has been useful in identifying genes of interest to the medical field and the biotechnology industry as well as in finding genes related to particular cellular functions. Additionally, creating a representative genomic library of an organism is a prerequisite for genomic mapping or complete genome sequencing. The success of a study involving genomic libraries is dependent upon the quality and features of the library. These features typically include the vector backbone used, the size of the genomic DNA insert, and the number of recombinant clones contained within the library.

To make a prokaryotic gene library, the complete bacterial chromosomal DNA is cut with a restriction enzyme and each of the fragments is inserted into a vector, usually a simple ColE1-derived plasmid. This mixture of vectors containing a different piece of the bacterial chromosome is transformed into a suitable bacterial host strain and a large number of colonies, each containing a single vector plus insert, are kept. These must then be screened for the gene of interest. If the gene has an observable phenotype, this may be used. Otherwise, more general methods such as hybridization or immunological screening are necessary.

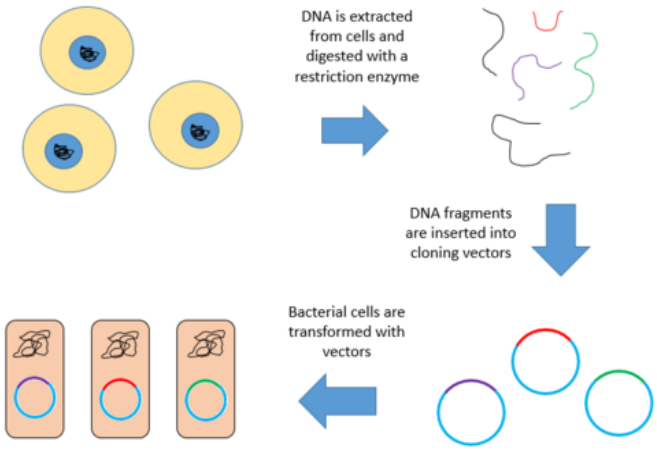


Figure 5.4: Construction of genomic library (source: Wikipedia.org)

10.10 BLOTTING TECHNIQUES: SOUTHERN NORTHERN AND WESTERN BLOTTING

Southern blotting:

Southern blot technique involves detection and immobilization of target DNA onto a membrane. This technique was invented in 1975 by E. M. Southern.

Steps:

1. The DNA fragments are first separated on an agarose gel and then transferred and immobilized onto a membrane.
2. After the gel has been run, the DNA fragments are denatured (i.e. the strands are separated) using strong alkali.
3. As electrophoretic gels are fragile, the DNA in them can diffuse within the gel, it is usual to transfer the denatured DNA fragments by blotting onto a nitrocellulose membrane.
4. The single-stranded nature of the DNA on the membrane is important to allow complementary DNA sequences to be able to bind to the DNA fragments attached to the membrane.
5. The transfer process can be mediated either electrophoretically or through capillary attraction by placing the gel and membrane in contact with each other and allowing the buffer to flow through the gel onto the membrane – the DNA fragments move with the buffer and become trapped on the membrane. Initially, nitrocellulose membranes were used, but these were fragile and easily broken. Nylon membranes are commonly used today.
6. After transfer, the DNA fragments need to be fixed to the membrane so that they cannot detach. A number of methods of fixing are available including baking at 80°C and ultraviolet cross-linking. UV cross-linking is based on the formation of crosslinks between a small fraction of the T residues in the DNA and the positively charged amino groups on the surface of the nylon membrane.
7. Following fixation, the membrane is placed in a solution of labelled (often radioactive) single-stranded nucleic acid – either single-stranded DNA or RNA.
8. The labeled nucleic acid (or **probe**) is allowed to hybridize to its complementary partner sequence on the membrane.
9. The interaction between the single-stranded probe and its complementary sequence will result in the binding of the probe to the membrane through non-covalent hydrogen bonding that normally holds the DNA double helix together.

10. The membrane is then washed extensively to remove non-specifically bound probes, and specific interactions are detected by exposing the membrane to X-ray film. Southern blotting is used to detect DNA sequences that are either identical or similar to the sequence of the probe.

Northern blotting

Northern blotting or Northern blot hybridization is a variant of Southern blotting in which the target nucleic acid is RNA instead of DNA. This method is used to measure the amount and size of RNA. The detection of RNA sequences is performed by the use of probes.

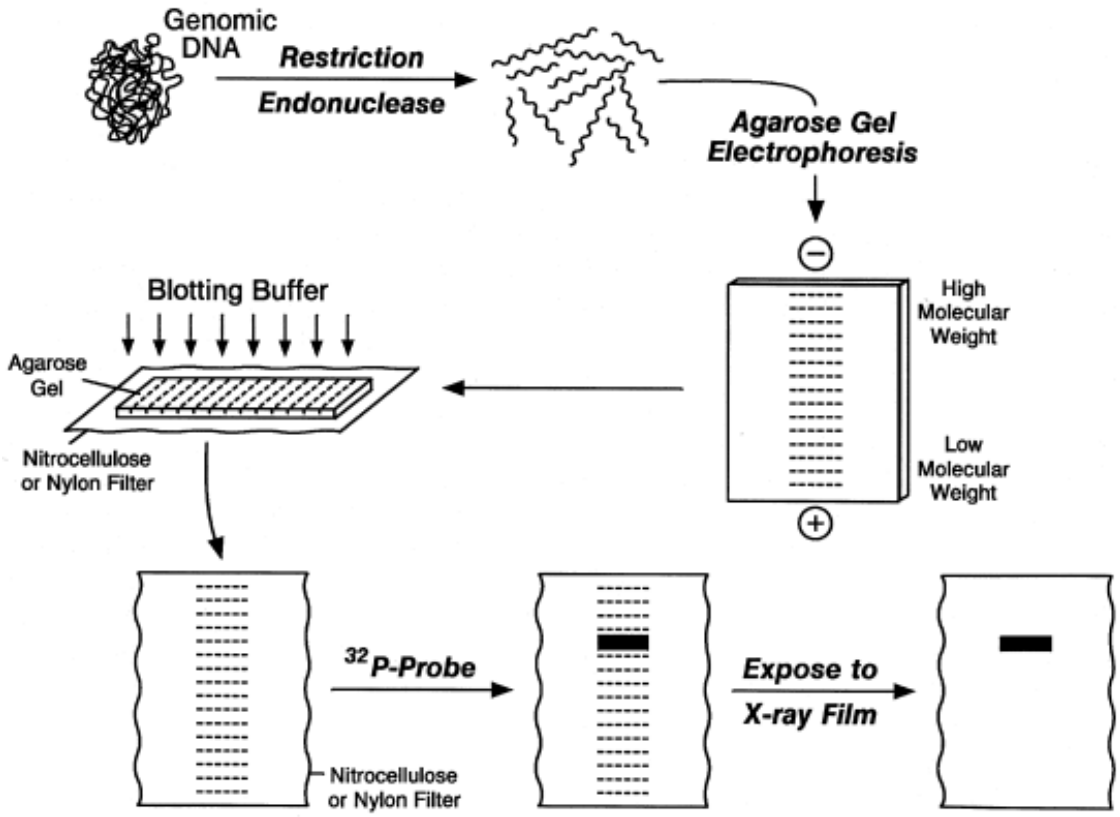


Figure 10.5: Steps involved in the Southern blotting (copyright MyBioSource)

Steps:

1. RNA extract is electrophoresed in an agarose gel, using a denaturing buffer to ensure that the RNA do not form inter or intramolecular base pairs.

2. After separation on an agarose gel, the gel is blotted onto a reactive DBM (diazo benzyloxymethyl) paper, and hybridized with a labelled probe.

3. RNA bands can also be blotted onto nitrocellulose paper under appropriate conditions and suitable nylon membranes.

4. Specific RNA molecules are then detected by hybridization using labelled single-stranded DNA or RNA sequences that are complementary to particular RNAs.

Nylon is a generic name for any long-chain synthetic polymer having recurring polyamide groups. Two types of nylon membranes are: unmodified (or neutral) nylon and charge-modified nylon which carries amine groups and is therefore, also known as positively charged nylon. Both types of nylons are able to bind with single and double stranded nucleic acids.

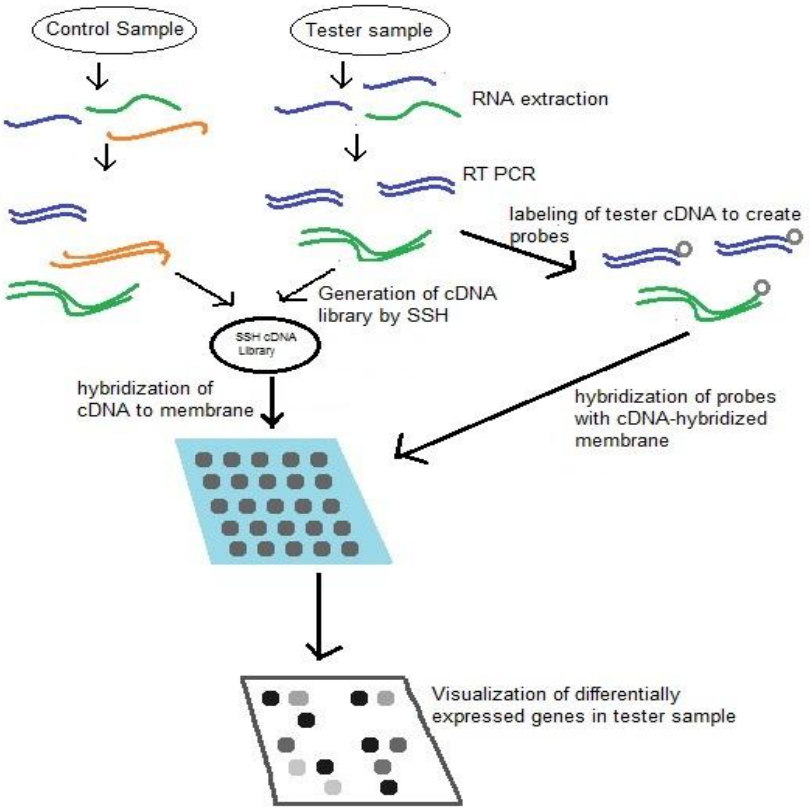


Figure 10.6: Northern Blot Hybridization (Slideshare.net

Western blotting

Western blotting is the method of detection of specific proteins using antibodies or immune blotting techniques. This technique requires antibodies against the test protein. Proteins are

separated through a polyacrylamide gel containing the detergent SDS to keep them in an unfolded (**denatured**) state.

Steps:

1. The proteins are transferred from the gel onto a membrane in much the same way as Southern blotting.

2. Particular proteins are then detected using antibodies. The specific interaction between the antibody and its antigen occurs on the membrane, and the position of the bound antibody is detected.

3. Initially radio-labelled antibodies were used, but these have been largely superseded by antibody 'sandwiches'.

4. The sandwiches work through the binding of one unlabelled antibody (the primary antibody) to the antigen on the membrane.

5. A second, labeled, antibody (the secondary antibody) is then used to detect the presence of the first antibody.

This has several advantages; firstly, the multivalent nature of antibody binding means that a substantial increase in sensitivity is achieved, and secondly, a single secondary antibody can be used to detect a number of different primary antibodies.

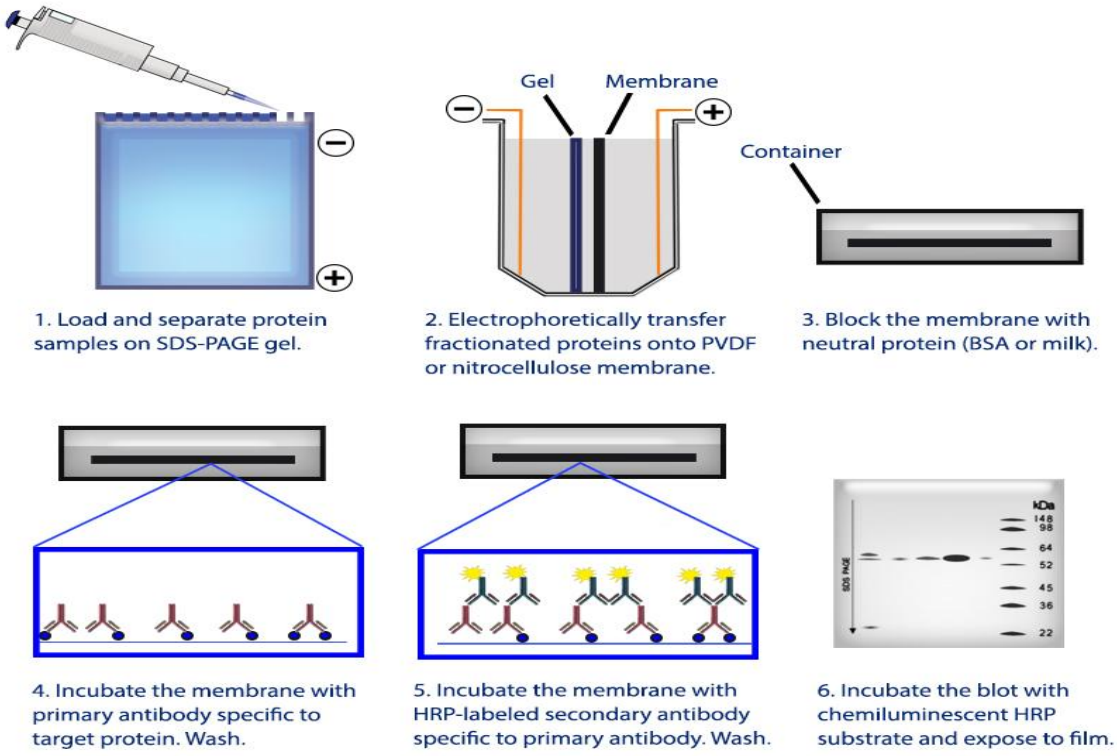


Figure 10.7: Western Blot Hybridization (Source: creative Biolabs)

10.11 SUMMARY

The basic process of recombinant DNA technology revolves around the activity of DNA in the synthesis of protein. By inserting genes into the genome of an organism, the scientist can induce the organism to produce a protein it does not normally produce. Gene cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. Restriction enzyme, also called restriction endonuclease, is a protein produced by bacteria that cleaves DNA at specific sites along the molecule. After introduction of recombinant DNA into the host cell it is essential to identify those cells which received the recombinant DNA molecule, hence selection of recombinant and screening is performed by colony hybridization, blue white screening and by positive selection methods. Hybridization is the process of combining two complementary single-stranded DNA or RNA molecules and allowing them to form a single double-stranded molecule through base pairing. Hybridization is a part of many important laboratory techniques such as polymerase chain reaction and Southern blotting. A gene probe (nucleic acid probe) is a single-stranded nucleic acid fragment that interacts with a complementary sequence of a target nucleic acid. The use of DNA fingerprinting depends upon the presence of repeating base sequences that exist in the human genome. The repeating sequences are called restriction fragment length polymorphisms (RFLPs). As the pattern of RFLPs is unique for every individual, it can be used as a molecular fingerprint. A genomic library is a collection of the total genomic DNA from a single organism. Blots are techniques for transferring DNA, RNA and proteins onto a carrier so they can be separated, and often follows the use of gel electrophoresis. Southern blot is used for transferring DNA and northern blot is for transferring RNA and western blot is used for protein.

10.12GLOSSARY

Amino acid: The constituent subunits of proteins. Amino acids polymerize to form linearchains linked by peptide bonds; such chains are termed polypeptides (or proteinsif large enough). There are twenty commonly occurring amino acids of which allproteins are made.

Annealing: Spontaneous alignment of two complementary single polynucleotide (RNA, orDNA, or RNA and DNA) strands to form a double helix.

Anticodon: Triplet of nucleotide cases (codon) in transfer RNA that pairs with (is complementary to) a triplet in messenger RNA. For example, if the codon is UCG, theanticodon might be AGC.

AntisenseRNA: RNA produced by copying and reversing a portion of an RNA-encodingDNA, usually including a protein-specifying region, and placing it next to atranscription-control sequence. This cassette can be delivered to the target cell,resulting in genetic transformation and production of RNAthat is complementary to the RNA that is produced from the original, not-reversed, DNAsegment. This complementary, or antisense, RNA is able to bind to the complementary sequences of the target RNA, resulting in inhibition of expression of thetarget gene.

Base: On the DNA molecule, one of the four chemical units that, according to theirorder, represent the different amino acids. The four bases are: adenine (A),cytosine(C), guanine (G), and thymine(T). In RNA, uracil (U) substitutes forthymine.

Basepair: Two nucleotide bases on different strands of a nucleic acid molecule that bondtogether. The bases generally pair in only two combinations; adenine withthymine (DNA) or uracil (RNA), and guanine with cytosine.

Bacillus thuringiensis (Bt): A naturally occurring microorganism that produces a toxin protein that only killsorganisms with alkalineing stomachs, namely such as insect larvae. As a Whendelivered as a part of the whole killed organism, this toxin protein has beenused for biological control for decades. The genetic information that encodesthe toxin protein was identified and moved into plants to make them insecttolerant.

Biotechnology: Development of products by a biological process. Production may be carried out by using intact organisms, such as yeasts and bacteria, or by using natural substances (e.g. enzymes) from organisms.

Deoxyribonucleic acid (DNA): The molecule that carries the genetic information for most living systems. TheDNA molecule consists of four bases (adenine, cytosine, guanine, and thymine)and a sugar-phosphate backbone, arranged in two connected strands to form adouble helix. A term often used to describe the configuration of the DNA molecule. The helixconsists of two spiralling strands of nucleotides (a sugar, phosphate, and base),joined crosswise by specific pairing of the bases. See also Deoxyribonucleic acid;Base; Base pair.

Endonuclease: An enzyme that breaks nucleic acids at specific interior bonding sites; thusproducing nucleic acid fragments of various lengths.

Enzyme: A protein catalyst that facilitates specific chemical or metabolic reactionsnecessary for cell growth and reproduction.

Escherichia coli (E. coli): A bacterium that inhabits the intestinal tract of most vertebrates. Much of thework using recombinant DNA techniques has been carried out with this organismbecause it has been genetically very well characterized.

Eukaryote: A cell or organism containing a true nucleus, with a well-defined membranesurrounding the nucleus. All organisms except bacteria, archebacteria, viruses,and blue-green algae are eukaryotic. Cf. Prokaryote.

Exonuclease: An enzyme that breaks down nucleic acids only at the ends of polynucleotidechains, thus releasing one nucleotide at a time, in sequential order.

Gene therapy: The replacement of a defective gene in an organism suffering from a geneticdisease. Recombinant DNA techniques are used to isolate the functioning geneand insert it into cells. Over three hundred single gene genetic disorders havebeen identified in humans. A significant percentage of these may be amenable togene therapy.
Genetic code: The mechanism by which genetic information is stored in living organisms. Thecode uses sets of three nucleotide bases (codons) to make the amino acids that, inturn, constitute proteins.Genetic engineeringA technology used to alter the genetic material of living cells in orderto make them capable of producing new substances or performing newfunctions.
Genetic Map: A map showing the positions of genetic markers along the length of a chromosomerelative to each other (genetic map) or in absolute distances from each other**Genetic screening:** The use of a specific biological test to screen for inherited diseases or medicalconditions. Testing can be conducted prenatally to check for metabolic defectsand congenital disorders in the developing foetus as well as postnatal to screenfor carriers of heritage diseases.

10.13 TERMINAL QUESTIONS AND ANSWERS

Question1. What is recombinant DNA technology? What are the basic steps involved in recombinant DNA technology?

Question2. Write short notes on the following:

a) Ligase

b) Linkers

c) Adapters

d) cDNA

Question3. Write a note on DNA fingerprinting and the steps involved in it?

Question4. What are molecular markers in recombinant DNA technology?

Question5. Write note on following:

a) RFLP

b) RAPD

c) AFLP

Question6. Elaborate the following techniques:

a) Southern blotting

b) Northern blotting

c) Western blotting

7. Plasmids are used in genetic engineering because they:

a) are easily available

b) can replicate

c) can integrate with host's chromosome

d) are inert

8. Which of the following enzyme is used to join bits of DNA?

a) Ligase

b) Primase

c) DNA polymerase

d) endonuclease

9. The sites of DNA where restriction enzymes act are generally

a) Palindromic

b) Tandem repeats

c) CG rich regions

d) TATA Boxes

10. Disease causing organisms are identified by using

a) MAb's

b) Microprojectiles

c) DNA probes

d) Gene transfer

10.14 REFERENCES

1. T. A. Brown. Gene Cloning and DNA Analysis: AN Introduction, 8th edition.

2. David P. Clark and Nanette J. Pazdernik. Biotechnology: Applying the genetic revolution

3. Sandy B. Primrose, Richard. Principles of gene manipulation and genomics.

4. https://www.ndsu.edu/pubweb/~mcclean/plsc731/mapping/mapping1.htm

5. https://www.nature.com/scitable/topicpage/restriction-enzymes-545/

6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4287216/

UNIT 11: CLONING VECTORS

CONTENTS

11.1 OBJECTIVES

After studying this module, you shall be able to learn and understand:

i. Basic concept of cloning
ii. Cloning vectors and their desirable properties
iii. Types of cloning Yeast
iv. *E. coli*
v. pBR 322

11.2 INTRODUCTION

The term cloning in recombinant technology refers to the production of 'clones' or genetically identical organisms. In natural cloning, the asexual reproduction methods are used by bacteria, fungi and plants to produce genetically identical offspring. A number of organisms like fungi, algae, protozoan and plants produce clones naturally through asexual reproductions that are genetically identical to the parent organism. Using processes like binary fission, budding, fragmentation and parthenogenesis organism can produce genetically identical offspring. Formation of identical twins in case of humans is also natural cloning.

Different Types of Cloning:

1. Molecular Cloning/ DNA Cloning/ Gene Cloning: Molecular cloning involves the production of large quantities/ copies of gene or DNA fragments. Molecular cloning involves the construction of recombinant DNA by insertion of gene of interest in vector DNA. Several copies of this constructed recombinant DNA are produced after it is introduced into host cells and replicates as the host cells proliferate. Finally either the gene or expressed protein is extracted, purified and sequenced. Commonly, these vectors belong to bacteria, yeasts, viruses, or plasmids.

2. Reproductive Cloning: Reproductive cloning is used for animals cloning. In reproductive cloning involves a mature somatic cell is taken out from the animal that has to be cloned. After this the somatic cell DNA is transferred into an enucleated egg cell (produced by removing the nucleus). The cell division in the restructured egg is stimulated using electric current. After few cell divisions the restructured egg develops into an embryo. The embryo is then implanted into the womb of a healthy adult female where it will develop until birth. The genetic makeup of this young animal is similar to the somatic cell donor animal and is

therefore known to be the clone of the donor animal. Dolly, the clone sheep was also created using reproductive cloning by Ian Wilmut in 1996. There are enormous potential benefits of this cloning method in medicinal and agricultural fields.

3. Therapeutic Cloning/Embryo Cloning: Therapeutic cloning essentially has the same process as reproductive cloning, however, with distinct outcomes and objectives. The objective of therapeutic cloning is to learn and understand human development and cure disease by production and use of stem cells. Stem cells can be used for the production of different types of specialized cells in the human body. Embryonic stems can be extracted out and used to produce specialized cells. Ethical concerns have been raised as extraction of stem cells cause the embryo destruction. But owing to the potential and crucial role of these stem cells in treating different diseases and also in replacing damaged cells to cure injuries, scientists and researchers find extreme hope in therapeutic cloning or embryo cloning.

11.3 PLASMID BIOLOGY

Plasmids may carry from half dozen to several hundreds of functional genes. The limiting features of plasmids are that they can multiply only within a host cell. Mostly plasmids are carried by bacteria and are essential part of 50% of bacteria found on earth. In some higher organisms also these plasmids are found as extra chromosomal segments like yeast and fungi. One of the best examples of higher organism's plasmid is 2m circle, a well known cloning vector of yeast origin. The number of plasmids found in bacteria per chromosome is known as the copy number of that plasmid, mostly bacteria have one or two copy number, but in some cases they may carry a copy number of 50 to hundreds, these are called high copy number plasmids. Similarly the size of plasmids is also enormously variable and it varies from few hundred base pairs to thousands of base pairs. Mostly plasmids carry genes which are responsible for their own maintenance, but some plasmids attribute characters to the host cells. In molecular biology, we use a number of genetically engineered plasmids to carry the genes for tailoring of genetic information, and other purposes.

Plasmids are circular, double stranded DNA (dsDNA) molecules, found free in host cells, and these are additional DNA than chromosomal DNA. The occurrence of these extra chromosomal DNAs naturally as parasitic or symbiotic relationship in bacteria and some lower eukaryotic cells like yeast. The distribution of plasmids in each daughter cell is performed just like chromosomal DNA, these plasmids replicate and segregate themselves to distribute equally. Plasmids are naturally occurring extra chromosomal DNA found in various

groups of bacteria. These plasmids have capability to self replicated due to presence of origin of replication site in it. The size of plasmid DNA ranges from 1 kb to 250 kilo base pairs. In nature plasmids have very important role in bacterial evolution. Almost all plasmids are double stranded circular DNA, conferring an extra phenotypic character to the bacteria. There are various functions which are served by these independent molecules. These characters may include antibiotics resistance (ampicillin, tetracycline, kanamycin etc.), abiotic stress tolerance (heat, cold, salt, toxic etc.) or may responsible for synthesis of some special polypeptides (toxins, metabolites). The closed-circular DNA of plasmid is cross coiled over its own axis in three-dimensional space to form a Super coil. Plasmids can be classified on the basis of various features found in it.

- Fertility F-plasmids, having a tra gene which enables bacterium to conjugation through sex pili.
- Resistance (R) plasmids, containing antibiotics or poisons resistant genes, conferring antibiotics resistance to the host bacterium also known as R-factors.
- Col plasmids are genes that transcribed and translated for bacteriocins, these proteins are responsible for killing of other bacteria.
- Degradative plasmids, provide the capability to bacterium for degradation of various organic/inorganic substances for harmful for bacteria, e.g. toluene and salicylic acid.
- Virulence plasmids, confers pathogenicity to the bacterium.

Plasmids having resistance and Defense related mechanisms:

- Antibiotic resistance for aminoglycosides, b-lactams, chloramphenicol, sulfonamides, trimethoprim, fusidic acid, tetracyclines, macrolides, fosfomycin
- Heavy metal ions resistance for Ni, Co, Pb, Cd, Cr, Bi, Sb, Zn, Cu and Ag
- Tolerance to mercury and other mercury compounds
- Toxic anions resistance such as chromate, selenate, tellurite, arsenate, arsenite, borate, etc
- Intercalating agent resistance such as acridines and EtBr
- Radiation like UV and X-rays damage protecting plasmids
- Bacteriophage DNA restriction systems
- Resistance towards some bacteriophages

Virulence related plasmids:

- Bacteriocins synthesizing plasmids
- Antibiotics synthesizing plasmids
- Crown gall tumors and hairy root inducing plasmids
- Nodulation in legumes related plasmids

Metabolic Pathways:

- Solbulization of sugars like lactose, raffinose, sucrose related plasmids
- Biodegradation of aliphatic and aromatic hydrocarbons
- Biodegradation of halogenated hydrocarbons like polychlorinated biphenyls
- Bioegradation of proteins
- Hydrogen sulfide Synthesising Plasmids
- Alcaligenes Denitrification
- Pigment synthesising plasmids

Fertility F-plasmids: With the discovery that genetic recombinants can be done by mixing particular Escherichia coli K 1 2 strains together, it was realized that the reason behind getting recombination is an iniderectional transfer of DNA segments from F- donor strains that contained an infectious "fertility factor," F. The F was found to replicate independently and inducts its DNA crossing the cell envelopes of bacteria which comes in contact. The injected DNA gets recombined with the chromosomal DNA at various locations. The F plasmid is covalently closed circular plasmid having approximately 60 genes with a total length of 100 kilo bases.

Resistance (R) plasmids: The resistance in a bacterium attributed to various mechanisms such as chromosomal mutations, in chromosomal alterations the resistance is most commonly associated with extra chromosomal elements which are commonly acquired from other bacteria. These moving transposing elements may be plasmids, transposons, and/or integrons. The intrinsic mechanisms that evolves efflux pumps to out multiple kinds of antibiotics, are supposed to be major contributors to multidrug resistance. Bacteria can acquire antibiotic

resistance by any of the two mechanisms whether intrinsic or acquired mechanisms. The bacteria can have naturally occurring genes such as, AmpC, β-lactamase of gram-negative bacteria may have Intrinsic mechanisms in combination with efflux systems.

Col plasmids: Escherichia coli and some other bacteria produce Colicins, a toxic protein. The production of colicin involved in bacteria to bacteria competition and virulence determination. These colins are different in their activity to kill other bacteria though they share some common features like lethal colicin release, smililar genetic sequences having genes like a colicin, lysis and immunity related genes producing peptides that interacting with a specific locus in the colicin protein seuqence. These have immunity towards colicin but when colicin is produced by a cell, it dies. These colicin related gene clusters are carried by some special plasmids.

Degradative Plasmids: In our daily life microorganisms play a great role in degradation of various products and by products like sewage, oil waste, agricultural byproducts, various pesticides, toxic substances etc. Many microorganisms have capacity to break down complex organic molecules and ability to recycle them by including these in to their meabolic cycles exhibiting a variety of degradative functions. Bacteria like Pseudomonas, Alcaligenes, Chromobacterium, have plasmids which have different genes responsible for degradation of a variey of inorganic and organic substances, these are known as biodegrading bacteria.

Virulence plasmids: The bacteria can be subdivided into many groups on the basis of their pathagenicity, the pathogenicity of a bacterium mainly depends upon the presence or absence of pathogenic DNA sequences which are frequently associated with various pathotypes. In these bacteria the genetic information for pathogenicity have been acquired horizontally through plasmids, bacteriophages and genomic islands. These genomic rearrangements are utilized by bacteria for their evolution though variants efficient in rearrangements, excision and transfer for affinity to additional DNA for creation of new (pathogenic) variants.

Plasmids are stably-inherited extra chromosomal, autonomously replicating; double stranded and covalently linked circular DNA molecules. Their size varies from 5000 to 400000 bp. These are commonly found in bacteria and also archaea and eukaryotes. An ideal cloning vector should have the following three properties:

(i) **Low Molecular weight:** This aid in easy handling and isolation due to resistance to shearing also low molecular weight vectors are present in multiple copies. Transfer efficiency of low molecular weight vector is quite high.

(ii) **Origin of replication:** The vectors should have a compatible an origin of replication with the host. It is important to have a multiple copies of a vector inside the host.

(iii) **Selection markers:** These markers help in the selection process of the chimaeric molecules over self annealed plasmid molecules (like antibiotic resistance).

(iv) **Multiple Cloning Site (MCS)/ Polylinker:** Presence of MCS or polylinker, which contains many unique restriction sites on vector is important requirement as for cloning both the vector and gene of interest has to be digested with the same restriction enzymes and then are ligated using DNA ligase.

Plasmids carry their own origin of replication and therefore can be maintained stably in host cells. These plasmids can be introduced in host cells by transformation. The typical approach is to use a plasmid that carries a gene that is required for the growth of host cell under particular condition (s) like antibiotic resistance gene. Under these conditions only the transformed host cells carrying the recombinant molecule are able to grow in the pressure of that antibiotic. Such genes aids in the selection of cells containing the recombinant plasmid are known as marker/selection genes. A DNA fragment (insert) capacity of these plasmid vectors is up to 15000 bp. pBR322 and its derivatives of like pUC 18/19 are most commonly used plasmid vectors in cloning.

Plasmid vectors

To carry and replicate the DNA fragment for various purposes, a molecular vehicle is needed in the process of molecular cloning. The transfer of DNA fragments with the help of molecular cloning vectors became possible due their ability to self-replication in E. coli or any other host cell, autonomous replication are responsible for this feature. Thus the origin of replication plays an important role in the molecular cloning. Most cloning vectors were engineered originally from extra chromosomal elements found in nature such as bacteriophage and plasmids. Plasmids are the DNA molecules that can replicate itself inside a host cell. These molecular vectors are used for carrying cloned fragments of DNA. The vectors may be a small multi-copy plasmid or a designed and engineered virus. Almost all plasmid vectors are the engineered extra chromosomal naturally found plasmids, isolated from different types of bacteria. Naturally found plasmids have several limitations; for

example, some are stringent and not relaxed (pSC101), some has poor marker genes (ColE1), and some are too large (RSF2124). To overcome the limitations of natural vectors, artificial plasmid are designed and engineered by combining different elements. Artificial plasmids vectors are classified into two broad types based on their use:

1. Cloning vector

2. Expression vector

Apart from these two, there is another class of vectors known as shuttle vector. Shuttle vectors can be propagated in two or more different host species (both in prokaryotes and eukaryotes). Hence, inserted DNA can be manipulated and replicated in two different cellular systems. Cloning vectors are designed for efficient transfer of foreign DNA into the host. Expression vectors have efficient machinery for cloning and expression of foreign gene in the hostsystem. Selection of a vector depends upon various criteria decided by the experimental goal.

Plasmid Expression vector:

Once a recombinant DNA has been made into a vector, the expression of that gene may or may not take place. The proper expression of a gene the structural gene and the corresponding promoter should be cloned on the same segment. In specialized expression vectors these promoter genes are provided by the vectors, only the structural genes have to be inserted on proper places. The best example of it is the vectors having capability of blue white screening have lac promoter, in these the multiple cloning site lies just in the promoter region. The most important and exploit use of genetic engineering is to use recombinant microorganism for production of various biochemicals. These biochemicals are being used widely in different industries for well being of the human used in pharmaceutical industries, cosmetics, bio-plastics etc. These biochemical have different origins, but due to advantage of recombinant DNA technology we are able to produce in bacteria. The basic of phenomenon of this is, the over expression of genes by some modifications or rearrangements in the natural sequences. This technique of over expression of genes is also being used in various structural studies, in determining biological functions etc. As convenient Escherichia coli have been widely used as the prokaryotic host for expression and overproducing various proteins. Its advantages are:

(1) The genetics and physiology or E. coli is well characterized and arrays of expression vectors are present.

(2) The manipulation in E. coli is easily and cost efficient.

(3) The production of foreign protein can be achieved up to the level of 5%-30% or more of the total protein of the cells. In many instances, however, we cannot use E. coli. Like, for proteins that requires post translational modification. In the case of heterologous expression, it fails to produce polypeptide to assume its native configuration.

11.4 CLONING VECTOR

Cloning vectors are designed for efficient transfer of foreign DNA into the host. Expression vectors have efficient machinery for cloning and expression of foreign gene in the host system. Selection of a vector depends upon various criteria decided by the experimental goal.

One of the most requirements for cloning is a vector. A cloning vector is self replicating small piece of DNA of plasmid, virus, yeast or cell of higher organism into which gene of interest can be cloned and expressed. The vector and foreign DNA (insert) are cleaved to produce the staggered cut generating similar ends. Using DNA ligase these are then spliced together. This recombinant molecule is then introduced into host cells by transformation or transfection. Since the vectors have origin of replication recognizable by host cells manifold multiple copies of the foreign DNA can be produced. The most commonly used cloning vectors are the following: 1. Plasmids. 2. Bacteriophages 3. Bacterial artificial chromosomes (BAC). 4. Yeast artificial chromosome (YAC). 5. Mammalian artificial chromosome (MAC).

11.4.1 YEAST ARTIFICIAL CHROMOSOMES (YACS)

First described in 1983 by Murray and Szostak, a yeast artificial chromosome has sequences to exist inside E. coli as a circular plasmid and contains sequences to maintain as linear nuclear chromosome in yeast. The number of clones in a genomic library can be greatly reduced. YAC vectors have following elements:

1. coli origin of replication
2. Yeast origin of replication
3. Elements of eukaryotic yeast chromosome (centromere and telomere region)
4. Selection markers for both the hosts (Bacterial as well as Yeast)

5. Maintained as linear DNA-like chromosome.

6. Introduced into the yeast cells by electroporation.

YAC is a vector used to clone DNA fragments larger than 100 kb and up to 3,000 kb. YACs are useful for the physical mapping of complex genomes and for the cloning of large genes. Yeast artificial chromosomes are created placing a centromere(CEN), telomeres (TEL), and an autonomous replicating sequence (ARS) element together to replicate autonomously and conserve itself in yeast cells. ARS are supposed to function as initiation point for DNA replication in yeasts. A circular plasmid is restriction digested to create a lenear YAC vector. These YAC are cleaved in cented to creat two arms and the foreign DNA are ligated between these two arms forming a single linear molecule. TRP1 and URA3 genes are included in the YAC vector to provide a selection system for identifying transformed yeast cells that include

YAC by complementing recessive alleles trp1 and ura3 in yeast host cell.

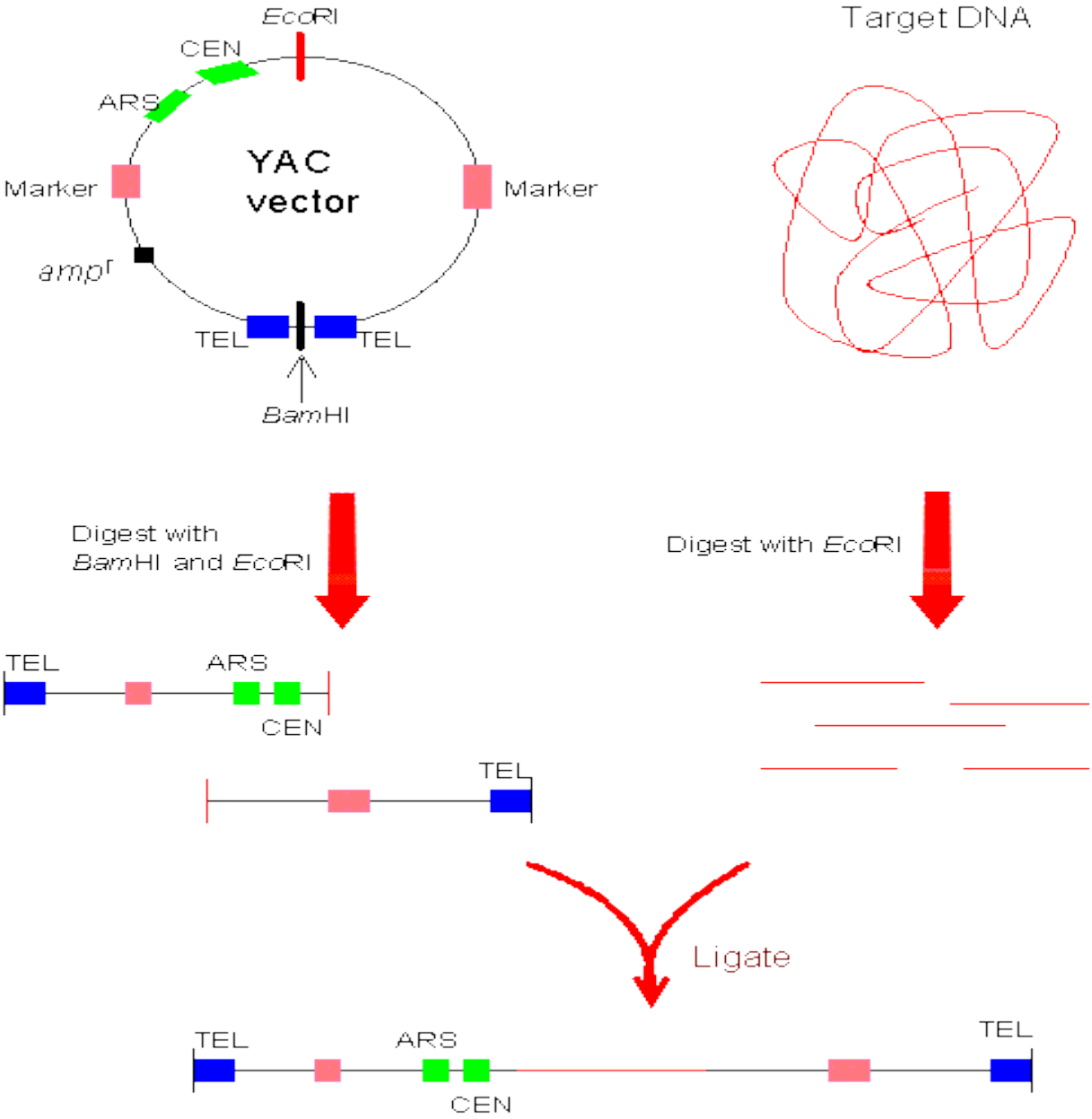


Fig 11.1 Cloning by using YAC vector.

Source:http://www.web-books.com/MoBio/Free/Ch9A4.htm

11.4.2 BACTERIAL ARTIFICIAL CHROMOSOMES (BACS):

Bacterial artificial chromosomes (BACs) cloning vectors are based on the fertility factor (F factor) and developed in early 1990s by Mel Simmons and coworkers. BACs plasmids are constructed for the cloning very long DNA fragments (typically 100,000 to 300,000 bp). They carry origin of replication and specific marker like chloramphenicol (CmR) resistance gene.Bacterial Artificial Chromosomes were prepared during human genome project for

preparation of large insert libraries having a cloning capacity of about 300 kbps. During the human genome project BACs got popularized due their high cloning capacity and easy maintenance. Usually BACs are maintained as single copy plasmids in per host cells and stable as such over hundreds of generations. For partitioning basically three genes are responsible, namely parA, parB and parC. The BACs can be maintained only in bacterial host deficient in homologous recombination (means it should have recA- gene)The total size of a BAC is about 7.4 kb and is introduced through electroporation in to the host cells. A single copy F-plasmid origin of replication (ori) is found in BAC.

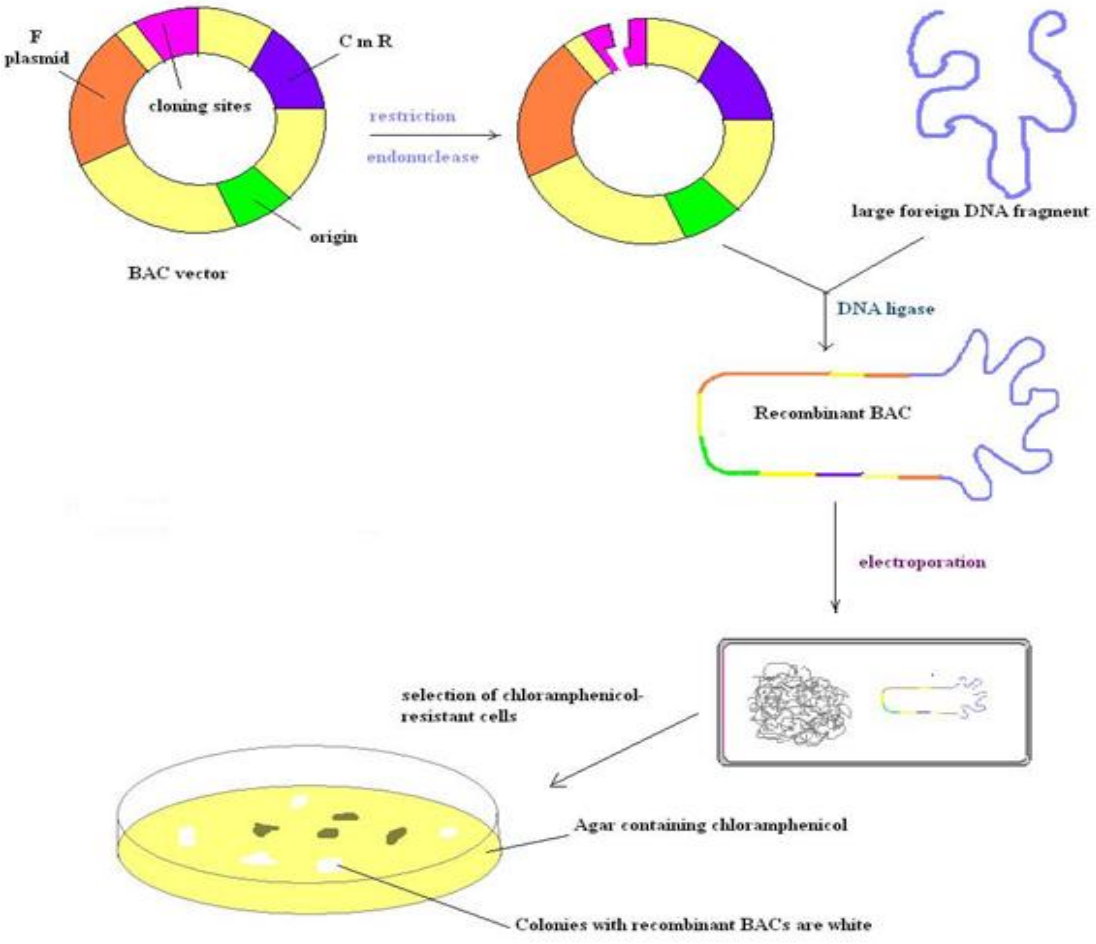


Fig.11.2 BACs as a Cloning Vector

The F (fertility) plasmid is relatively large and vectors derived from it have a higher capacity than normal plasmid vectors. F-plasmid has F (fertility) factor which controls the replication and maintain low copy number. Also conjugation can take place between F+ bacteria (male)

and F- bacteria (female) to transfer F-plasmid via pilus. Common gene components of a bacterial artificial chromosome are:

- oriS, repE – F for plasmid replication and regulation of copy number.
- parA and parB for maintaining low copy number and avoiding two F plasmids in a single cell during cell division.
- A selectable marker for antibiotic resistance; some BACs also have lacZ at the cloning site for blue/white selection.
- T7 and Sp6 phage promoters for transcription of inserted genes.

Bacterial Artificial Chromosomes are Bacterial cloning vector (derived from F plasmid) that can accommodate up to 350 kb (most commonly 120–150 kb) DNA sequences, and has a considerably lower error rate than the still larger capacity yeast artificial chromosome (YAC). BACs usually exist in a single copy per cell. Random BACs are selected at random from a genomic library and are then shotgun-sequenced. Most BAC vectors lack selectable markers suitable for mammalian cell selection but can be retrofitted by employing the Cre/loxP site specific recombination system.

For BACs to be used as cloning vehicle, the vector is linearized using restriction end nucleases, treated with phosphatase and then ligated with insert DNA fragment. After the recombinant DNA is produced it is introduced in E. coli host cells.

11.4.3 pBR 322

Plasmids are used as vectors to clone DNA in bacteria. One example of a plasmid used for DNA cloning is called pBR322 Plasmid. The pBR322 plasmid contains a gene that allows the bacteria to be resistant to the antibiotics tetracycline and amipicillin. To use pBR322 plasmid to clone a gene, a restriction endonuclease first cleaves the plasmid at a restriction site. PBR322 plasmid contains three restriction sites: PstI, SalI and ecoRI. The first two restriction sites are located within the gene that codes for ampicillin and tetracycline resistance, respectively. Cleaving at either restriction site will inactivate their respective genes and antibiotic resistance. The target DNA is cleaved with a restriction endonuclease at the same restriction site. The target DNA is then annealed to the plasmid using DNA ligase. After the target DNA is incorporated into the plasmid, the host cell is grown in a environment containing ampicillin or tetracycline, depending on which gene was left active. Many copies of the target DNA is created once the host is able to replicate.

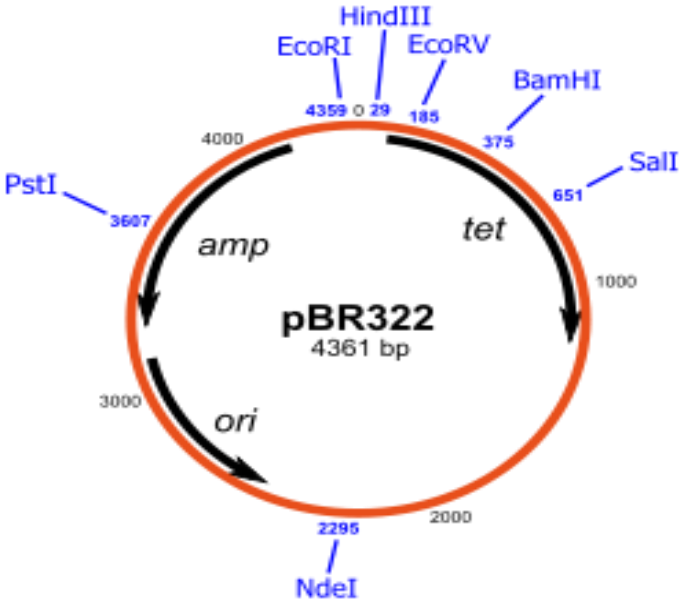


Fig.11.3 pBR322 Plasmid

pBR322 was one of the first plasmids used for the purpose of cloning. It contains genes for the resistance to tetracycline and ampicillin. Insertion of the DNA at specific restriction sites can inactivate the gene for tetracycline (an effect known as an insertional inactivation) or ampicillin resistance.

11.5 SUMMARY

Cloning in recombinant technology refers to the production of 'clones' or genetically identical organisms. Cloning vector is self replicating small piece of DNA of plasmid, virus, yeast or cell of higher organism into which gene of interest can be cloned and expressed. The most commonly used cloning vectors are: Plasmids, Bacteriophages, Bacterial artificial chromosomes (BAC), Yeast artificial chromosome (YAC), Mammalian artificial chromosome (MAC). Molecular vectors are used for carrying cloned fragments of DNA. The vectors may be a small multi-copy plasmid or a designed and engineered virus. We also learned about various. Key steps for cloning in plasmid vectors, Bacteriophage λ and derived vectors, Insertion vectors, Replacement vectors, Lysogenic pathway, Lytic pathway, In-vitro packaging of λ DNA with the use of helper phage, and about various Bacteriophage λ derived cloning vectors.Plasmids are stably-inherited extra chromosomal, autonomously replicating; double stranded and covalently linked circular DNA molecules. To identify the clones containing the gene of interest screening is carried out. Selection of clones carrying gene of interest is done on the basis of marker genes and or a reporter gene present on the vector used in cloning.

REFERENCES

- Cooke H. 2001. Mammalian artificial chromosomes as vectors: progress and prospects; Cloning Stem Cells, 3(4): 243-249.
- Dente L., Cesareni G., Cortese R. 1983. pEMBL: a new family of single stranded plasmids; Nuc. Acid Res. 11 (6) 1645-1655.
- Hall B.G. 2004. Predicting the evolution of antibiotic resistance genes. Nat Rev Microb 2 (5): 430–435.
- http://bioinfo2010.wordpress.com/2009/07/08/vector-bacteriophage-lambda-and-m13-7thapril/
- Kim et al. 1992. Stable propagation of cosmid-sized human DNA inserts in an F-factor based vector. Nucleic Acids Res. 20 (5): 1083–1085.
- Kim et al. 1995. Construction and utility of a human chromosome 22-specific Fosmid library; Genetic Analysis: Biomol Eng 12 (2): 81–84.
- Brown T.A. 2010. Gene cloning and DNA analysis: an introduction (6th edition); Willey Blackwell Ltd.
- https://epgp.inflibnet.ac.in/Home/ViewSubject?catid=t5vt4STquHRj94mcOBMr5g==
- Clark, David P., and Nanette J. Pazdernik. Molecular biology. Elsevier, 2012.
- Griffiths A.J.F, Miller J.H., Suzuki D.T, Lewontin R.C., Gelbert W.M. 2000. An Introduction to Genetic Analysis (7th edition); New York: W. H. Freeman.
- Robertis E.D.P.De, Robertis E.M.F. De. 2010. Cell and Molecular biology (8th edition); Lippincott Williams and Wilkins.

11.6 TERMINAL QUESTIONS AND ANSWERS

Question No.1 Explain the Plasmid Biology in detail?

Question No.2 what do you understand by the Cloning Vectors?

Question No.3 Write a short note on *pBR322 Plasmid?*

Question No.4 Explain in detail the Yeast Artificial Chromosomes?

Question No.5Explain in detail the Bacterial Artificial Chromosomes?

Unit 12 ANIMAL BIOTECHNOLOGY AND ITS APPLICATION

CONTENTS

12.1*OBJECTIVES*

In this unit students will learn:

- Details of the cell, organ and whole embryo culture
- The mechanism of In vitro fertilization.
- About the transgenic animal and gene therapy

12.2 *INTRODUCTION*

Biotechnology is technology based on biology and it utilizes the scientific and engineering principles to produce products by biologic agents to provide goods and services. Biotechnology offers new tools for improving health and productivity of organisms. It helps in detection, treatment and prevention of diseases. Biotechnology helps in various sectors of agriculture by improving overall health, breed and productivity of livestock. Biotechnology helps in improving food quality by introducing desirable traits through new genes into farm livestock and poultry. Biotechnology helps in the improvement of Farm animals and their feeds which results in reduction of animal wastes and minimizing the impact on the environment. Artificial insemination, embryo transfer, in vitro fertilization, genetic mapping and cloning techniques help in breeding programs. As a result of high demand for meat and the degradation of agricultural land, biotechnology is providing new methods to improve productivity in animal agriculture. Genetically engineered poultry, swine, goats, cattle, and other livestock also are starting to be used as 1) producers of pharmaceutical and other products 2) potential sources for replacement organs for humans and 3) models for human disease. Animal-made pharmaceuticals (AMPs) change biotech animals into "factories" to yield therapeutic proteins in their milk, eggs, and blood. Biotechnology can be used to produce human-compatible transplant organs, tissues and cells in pigs that can be vital to improving human health. Various methods are utilized to produce transgenic animals. Reproductive and cloning techniques provide the possibility of preserving the genetics of endangered species. The development in the field of biotechnology has so many advantages but the advancement in the field raises various concerns also. The biological technologies and other advances are yet to be considered and their use in the field of agricultural and biomedical work causes concerns regarding the safety of end products consumption, its undesirable effects on the environment, and its adverse effects on animal welfare.

12.3 *CELL, ORGAN AND WHOLE EMBRYO CULTURE*

CELL CULTURE

Cell culture refers to laboratory methods that enable the growth of cells in physiological conditions in vitro. The cell culture technique originated in the 20th century and it was used to 1) study growth and maturation of tissue 2) particular gene role in disease and metabolism 3) for large-scale production of biopharmaceuticals by hybrid cell lines and 4) studies of viruses and vaccine development. There are various experimental applications of cultured cells e.g. creating model systems to study basic cell biology, replicate disease mechanisms, or investigate the toxicity of novel drug compounds. Cell culture helps in manipulating genes and molecular pathways for research purposes. Cell culture leads to production of clone cell populations or specific cell types and well-defined culture systems which help in removing interfering genetic or environmental variables, this help in generation of high reproducible and consistent data that cannot be produced with whole organ systems study.

Safety protocol in Cell Culture Laboratory

Lab coats, gloves, and goggles should be worn to ensure protection of laboratory workers from potentially hazardous sources. When working with cell lines and biohazardous agents, personal protective equipment (PPE) must be worn in the cell culture lab. Culture work should be done inside biosafety cabinets which provide a steady, unidirectional flow of HEPA-filtered air and create an enclosed, ventilated workspace. Researchers should read the Material Safety Data Sheet (MSDS) of a regent before using it. Researchers should be well trained in handling various instruments and procedures.

Equipment used for cell culture

Numbers of equipment are required for the cell culture experiment and they provide desired conditions to carry out the cell culture experiment. The cell culture process required sterile conditions, so a separate space should be dedicated to this work. Various equipment can aid in achieving such a sterile workspace.

1) Biosafety cabinet:- To provide sterile work surface
2) Humid CO_2 incubator:- To create physiological environment for cellular growth
3) Inverted light microscope:- To evaluate cell morphology and count cells
4) Fridge, freezers (−20°C, −80°C), liquid nitrogen storage :- To store cells, cell material, and culture components
5) Centrifuge :- Help in cell condensation

6) pH meter:- To determine the correct pH of media components
7) Pipettes and pipettors:- To aliquot different volumes
8) Cell media and supplementary components:- To culture cells in desirable components
9) Hemocytometer:- To count cells, determine growth kinetics and prepare suitable plating densities
10) Autoclave:- To sterilize pipettes and other equipment in contact with cells
11) Vacuum pump:- To aspirate cell culture medium
12) Water bath: - To provide desired temperature.
13) Cell culture dishes:- To culture cells in different formats
14) Containers for waste :-To correctly dispose of waste

Aseptic Cell Culture procedure

Any kind of contamination can result in damage to cell culture and abnormal results leading to wrong scientific interpretations. Various precautions are taken to reduce the contamination of cell culture.

1) The air is full with microparticles which have the ability to cause infection, So all work should be done in a biosafety which restricts nonsterile aerosols and airborne components from contaminating cultured cells.
2) The biosafety cabinet should be kept in that part of the laboratory that does not obstruct its airflow by external sources of wind.
3) Biosafety cabinets should be sterile using UV light and the work surface should be decontaminated with an antifungal detergent (e.g., 5% Trigene) followed by 70% ethanol.
4) All equipment used inside the biosafety cabinet should be sprayed and wiped with 70% ethanol.
5) Minimum number of items should be kept inside the biosafety cabinet,
6) All instruments (incubator, centrifuge, microscope, water bath, fridge, and freezer) and equipment should be kept clean and free of dust. All instruments should be properly maintained.
7) Laboratory should be properly clean.
8) Treatment solutions can be added to water baths to prevent the growth of microbes.
9) Disposable gloves should be worn during experiments which are sprayed with 70% ethanol. And gloves should be discarded after the experiment.

10) Clean lab coats should be worn during experiments and should be clean at hot temperatures on a regular basis.

Reagents and Media for Cell Culture

The commercially available media and supplementary cell culture products are generally supplied in sterile condition. In the laboratory the material used for the culture can be autoclaved. Use of antibiotics (e.g., Penicillin/Streptomycin) help in controlling bacterial growth in media bottles after opening and in cell culture vessels.

Contaminations

The contamination cannot be altogether prevented, so it is important to recognize early signs of contamination in order to prevent it. Contaminants are generally due to biological agent bacteria, fungi, viruses, and parasites. The biological contaminants should be avoided because they can change the phenotype and genotype of the cultured cell line by competing for 1) nutrients 2) synthesis of alkaline and acidic or toxic by-products and 3) the potential interference of viral components with the cell culture genome. Other contaminants are chemicals (e.g., plasticizers in cell culture vessels) or other cell types co-cultured in the lab.

Bacterial Contamination

The bacteria have a fast doubling rate and this not only leads to easy detection in cell culture supernatants shortly after infection, but also facilitates quick spread. Cell cultures contaminated by bacteria generally appear turbid in appearance. The high metabolic rates of bacteria can also alter the pH of the culture media and thus change the color of phenol red to yellow. Bacteria can be detected by using microscopic techniques. Mycoplasma infections are hard to detect so it is advisable to routinely test cultures for their presence using polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), or immunostaining .

Fungal Contamination

Yeast's cell can easily be detected at low microscope magnifications. The supernatants of yeast contaminated cell culture appear turbid and have a distinct smell. The pH of the culture remains stable during the initial stages of infection but it increases in high contaminant concentrations.

Viral Contamination

Viruses are small obligatory parasites and they are not visible in generic light microscopy. Detection of viral contamination is hard. Some viruses may cause morphological changes in

the cultured cells and other species may integrate into the cellular genome and alter the phenotype of the investigated cell line. Virus's contamination in cell cultures occurs due to the use of animal-derived cell culture products such as trypsin or fetal bovine serum. The presence of viral contaminants can be detected by PCR, ELISA, immunocytochemistry, or electron microscopy.

Removing contaminants

1) To eliminate the contamination regardless of the type of contamination identified, affected cell cultures should be removed from the cell culture room and discarded to prevent the spread of infectious agents to other cultures.
2) The source of contamination should be identified.
3) The culture media and other cell culture components that have been in contact with the contaminated cells should be disposed
4) The surfaces should be clean that come in contact with the contaminated vessel (e.g., incubator, biosafety cabinet, microscope, aspirator).

The Cell Line

Cells cultured can be classified into three different types: primary cells, transformed cells, and self-renewing cells.

Primary cells can be obtained directly from human tissue or fibroblasts can be isolated from skin biopsies and hepatocytes can be obtained from liver explants. These types of cells can be used in Biomedical and translational research because they very well represent their tissue of origin. The disadvantage associated with them is related to biosafety restrictions associated with their handling and their finite life.

Transformed cells can be produced either naturally or by genetic manipulation. They can be used to provide fast growth rates and stable conditions for maintenance and cloning. But their manipulated genotype may cause karyotypic abnormalities and non physiological phenotypes e.g., Chinese hamster ovary (CHO), HeLa, human umbilical vein endothelial cells (HUVEC)

Self-renewing cells have the ability to differentiate into a diversity of other cells types, while their self-renewing property allows for long-term maintenance in vitro e.g. embryonic stem cells, induced pluripotent stem cells, neural and intestinal stem cells. Cell lines can be obtained commercially.

To grow cells in vitro in a culture medium, it is necessary to provide a proper microenvironment. Cells can either grow in a suspension culture or attach to substratum.

Cells grown in suspension generally adopt spherical shapes, while adherent cells display spiked or polygonal morphologies.

The Cell Culture Medium

To grow a cell in vitro a proper cell culture medium is required. Medium contains nutrients (such as carbohydrates, vitamins, amino acids, minerals) growth factors and hormones. Cell culture medium should have a proper pH and cellular osmotic pressure. To provide physiologically relevant microenvironment a solid or semisolid growth substrate is used which allow cell–matrix anchoring and cell–cell interactions respectively. Numbers of cell culture medium compositions have been synthesized according to the requirements of specific cell types. Serum (fetal bovine serum) is added to basal media that already contain a standard formulation based on amino acids, vitamins, carbon sources (e.g., glucose), and inorganic salts. Serum provides 1) growth factors and hormones 2) acts as a carrier for lipids and enzymes and 3) helps in the transportation of micronutrients and trace elements.

Temperature, pH, CO_2, and O_2 Levels

The cell culture depends on the temperature and the desired temperature for culture is the body temperature of the species and the microenvironment from which the cultured cell types were taken. For human and mammalian cell lines the incubated temperature is 36–37°C, but for cell lines of cold-blooded animals a wider temperature is used (ranges between 15°C and 26°C). Maintaining pH level is also important and for most human and mammalian cell lines pH should be tightly controlled and kept at a physiological pH level of 7.2–7.4. Cell culture temperature is maintained by incubators. Glucose fulfills the cell's energy needs and its metabolism releases pyruvic acid, lactic acid, and CO_2. Since the pH level is dependent on the balance of CO_2 and HCO_3^- (bicarbonate), the addition of bicarbonate-based buffers to cell culture media can equilibrate the CO_2 concentrations. Variations in atmospheric CO_2 concentrations can also change the pH level. Cells should therefore be cultured in incubators which regulate CO_2 tensions to be adjusted to 5–7%.

Subculturing

As the time passes, the number of cells increases in the cell culture medium with depletion of nutrients, and release of the toxic metabolites. So, to expand and maintain a healthy cell culture, it is necessary to do a new culture with a subset of cells from the originating culture, removing toxic by-products, and replenishing nutrients with fresh medium. So, when the

available space in the cell culture vessel reaches ~80% confluency, then the cell should be transferred to a new vessel. This process is called “passaging”. To subculture cells, cultured cells are enzymatically digested or mechanically dissociated to remove them from their substrate. Then cells are washed with phosphate-buffered saline (PBS) with no Mg^{2+} and Ca^{2+} to remove dead cells and are incubated at 37°C with sufficient digestive enzymes or chelating agent to cover the monolayer (e.g., trypsin, dispase, collagenase, ethylenediaminetetraacetic acid (EDTA)). The dissociated cells are collected in a sterile Falcon tube and the collected cells seeded in new culture vessels at the desired concentrations. The growth of adherent cells depends on available surface area, but in suspension culture the rate-limiting step is the concentration of cells in the medium. So, it is necessary to monitor growth rates in suspension cultures over time.

The subculturing of suspension cultures can be done by aseptically removing one-third of the cell suspension solution and replacing the volume with prewarmed complete medium.The cells need to be concentrated for transfer to new cell culture vessels, freezing, or other experimental assays. For that the cell must be suspension is centrifuged at 300 ×g for 10 minutes. After discarding the supernatant, the cell pellet is resuspended in the desired medium through gently pipetting cells up and down three times. The cells are fragile so centrifuge should not be done at high speed or vigorous pipetting should be avoided.

Quantification of Cells and Determination of Cell Viability

During the process of cell culturing, handling and passing cells can die, but a specific amount of cells are required to start culture and perform assay, so it is important to distinguish between live and dead cells. Cell counting is also useful for assessing growth rates. Cells are generally cultured in the millions; the number of cells are first counted in a small volume and then extrapolated to the full cell volume.

Cryopreservation of Cells

The cell can be preserved by freezing with cryoprotective agents (e.g., glycerol or dimethyl sulfoxide (DMSO)) that can prevent the formation of harmful extra- or intracellular crystals. DMSO is toxic to personnel and cultured cells and therefore cannot be added to cells without prior dilution. Chemically protective gloves should be worn to safeguard personnel from the hazards of DMSO as its solutes can easily penetrate membranes, including the skin. Cells can be preserved in liquid nitrogen (<130°C) for years since all life processes cease at these temperatures.

Thawing Cryopreserved Cells

To recover cells, 10 ml of complete medium is prewarmed in a water bath. Then the frozen vial is immediately placed into a 37°C water bath until two-thirds of the contents are completely thawed. The vial is wiped with 70% ethanol and placed in a biosafety cabinet. Then prewarmed medium (1ml) is added dropwise to the partially thawed vial to minimize the osmotic stress imposed upon the cells when DMSO is diluted. The contents of completely thawed vial is added to the remaining 9 ml of complete medium and centrifuged at 300 × g for 3 minutes. After that the supernatant is discarded and the cell pellet can be washed once in medium to remove residual cryopreservatives. Cells are then resuspended in complete medium and transferred to a cell culture vessel. Cell attachment should occur within 24 hours.

Application of cell culture

1) Model Systems to study Health and Disease: Cell culture technique helps to study the various cellular functions of wild-type cells and diseased cells. The co-cultures can be used to study interaction and route of infection between wild-type cells and pathogenic agents. Cancer cell lines are used to study mechanisms of cancer induction and its treatment. Human-induced pluripotent stem cells (hIPSCs) help in studying various molecular mechanisms of a disease.

2) Helps in Drug Development and Drug Testing: Cell culture technique is used as a tool that can screen novel chemicals, cosmetics, and drug compounds for their effectiveness and evaluate drug cytotoxicity in specific cell types.
3) Production of biological important components: cell cultures can be used for the production of genetically engineered proteins, antibodies, hormones and biopharmaceuticals that can be isolated and used therapeutically.
4) Virology and Vaccine Production: Cell culture of mammalian cells provide a host for viruses to replicate which allow scientists to study their growth rates, development, and conditions required for their infectious cycle. Also, the attenuated viruses used in vaccines against polio, measles, chicken pox, rabies, and hepatitis B are produced in animal cell cultures.
5) Tissue Regeneration and Transplantation: hIPSCs, embryonic stem cells, and adult stem cells have the ability to regenerate and differentiate into specialized cell types that can help in tissues or organs replacement.
6) Genetic Engineering and Gene Therapy: cell culture helps in introduction of new genetic material into the nucleus of cultured mammalian cells to study expression of specific genes and their impact on cells.

ORGAN CULTURE

Cell culture has few limitations like it doesn't represent the in vivo responses due to lack of an appropriate micro environmental context of the responding cell types. So, organ culture provides an opportunity to better replicate the tissue microenvironment.

Procedure

1) Tissue for human organ culture (HOC) is taken to the laboratory as quickly as possible to minimize deterioration, preferably within minutes of collection.
2) Then the excess blood is removed from the sample by immersing tissue in sterile phosphate-buffered saline prior to transfer into a sterile petri-dish for dissection into $<1\ mm^3$ fragments using two sterilized carbon steel single-edged razor blades stuck together under a dissecting microscope). Multiple samples can be collected from various regions of a tissue e.g. kidney samples can be taken from the medulla through to the cortex.

3) The dissected fragments are immediately immersed in tissue culture medium in a sterile plate containing culture inserts, treated as indicated, or left untreated and maintained in a 37°C incubator for the desired time periods.
4) Culture conditions may need to be changed depending upon the tissue source.

Application of Organ culture

1) It can be used to study Inflammatory Diseases
2) It can be used to study Systemic Diseases
3) It can be used to reveal altered response and signaling pathways in disease
4) It can be used to study Organ Transplantation
5) It can be used to study Cancer
6) It can be used to study Therapeutics

EMBRYO CULTURE

Embryo culture is a procedure for the cultivating of an embryo under aseptic conditions on a culture medium. The aim of this technique is to improve the quality of embryos developed in the laboratory. The embryos are cultured for 2 to 3 days to reach the four-to-eight cell stage before transfer to the uterus. Premature replacement of the human embryo to the uterus may lead to low implantation rates associated with human In vitro fertilization (IVF). The preimplantation embryo has specific needs depending on the developmental stage and the improvements in culture media are due to better understanding of the environment of the oviduct and uterus. A culture medium is an external environment for the human embryo. The component of cultured media is selected carefully in proper concentration So, as to minimize the stress on cultured embryos. The better understanding of both the physiological changes in oviduct and uterus and the different metabolic requirements of the cleavage-stage and blastocyst-stage embryo led to the concept of stage-specific or "sequential" complex media.

Generally the culture media contain following components

1) Carbohydrates: Zygotes and subsequent cleavage stages favor pyruvate as the primary source of energy, while the eight-cell-stage embryo uses glucose. Glucose is the key anabolic precursor and it is involved in the synthesis of triacylglycerols and phospholipids, and acts as a precursor for complex sugars and glycoproteins. Glucose is also utilized by the pentose phosphate pathway

(PPP) to generate ribose moieties required for nucleic acid production.

2) Amino acids: Amino acids are essential for the development and they regulate the mammalian preimplantation. Before the embryonic genome expression, the embryo uses carboxylic acids and AA as energy sources. Certain AA also functions as biosynthetic precursor molecules, osmolytes, buffers of internal pH, antioxidants and chelators, especially for heavy metals. The nitrogen requirement of embryos specifically changes with different requirements. The seven non-essential AA and glutamine stimulate the development of the early cleavage embryo. However, the presences of 13 essential AAs have an inhibitory effect on blastocyst development and viability at an early stage. Amino acids in culture media spontaneously undergo breakdown and produce ammonium into the culture medium. Ammonium is toxic to the embryo and culture media protocols are used to remove the accumulated ammonium.
3) EDTA (Ethylenediaminetetraacetic acid): It is a ligand and chelating agent (ability to "sequester" metal ions). Metal ions after bonding with EDTA remain in solution but exhibit diminished reactivity.
4) Regulation of cell volume-osmolytes: It is necessary to maintain the specific osmotic pressure in the cell. The osmotic pressure of oviduct fluid is >360 mOsmol. Addition of extracellular organic osmolytes, such as glycine, betaine, proline, alanine and hypotaurine protects the preimplantation embryo against hypertonicity and increases embryo development.
5) Impact of pH and buffers: The pH range for embryo culture media is between pH 7.4 and 7.2. Culture media pH is regulated by the balance of CO_2 concentration, and by the concentration of bicarbonate in the media. The intracellular pH in human cleavage embryonic cells is pH=7.2. pH plays an important role in maintaining intracellular homeostasis. The most commonly used buffer is 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
6) Macromolecules: In culture media sources of macromolecules are proteins e.g. human serum albumin or synthetic serum. Both are added at concentrations of 5 to 20%. Albumin helps in maintaining the stability of cell membranes and chelate trace amounts of toxic components presented in culture water, media components and culture dishes. Other functions are capillary membrane

permeability and osmoregulation. Physiological alternative to albumin is the glycosaminoglycan hyaluronate (also called hyaluronic acid or hyaluronan).

7) Vitamins: They work as antioxidants in culture media containing glucose and phosphate and also help in prevention of loss during respiration and metabolic control. Examples of vitamins used in culture media are ascorbic acid, cyanocobalamin, folic acid and tocopherol.
8) Growth factors: Growth factors play an important role in growth and differentiation from the time of morula to blastocyst transition.
9) Antibiotics: Embryo culture media are routinely supplemented with antibiotics to prevent bacterial contamination e.g. penicillin, streptomycin and gentamycin.

12.4IN VITRO FERTILIZATION (IVF) TECHNOLOGY

The modern technologies provide the possibility of freezing the embryos and semen for their long term preservation. In the laboratory a needle is used to aspirate immature oocytes from the ovaries and the oocytes are placed in a culture containing hormones for one day to mature. When the oocytes reach a point midway through the second division of meiosis, they are fertilized with live sperm. In rare cases, single sperm or sperm head is used for fertilization, which is injected through the tough outer zona pellucida of the oocyte, either below the zona or directly into the cytoplasm (intracytoplasmic injection, or ICSI). The zygotes result after fertilization is cultured until the embryo reaches a more advanced stage of development. In humans, of course, these combined techniques form the basis of in vitro fertilization procedures. In vitro fertilization (IVF) is a complex series of events in which mature eggs are collected (retrieved) from ovaries and fertilized by sperm in a lab. Then the embryos are transferred to the uterus of a female. IVF is an assisted reproductive technology and done using a couple's own eggs and sperm or from donor. The success of this techniqueand the birth of a healthy baby depend on many factors, such as your age and the cause of infertility. IVF procedure is required when infertility occurs for any reasons like Fallopian tube damage or blockage, Ovulation disorders etc. The risk factors associated with IVF are Multiple births, Premature delivery and low birth weight, Ovarian hyperstimulation syndrome (due to use of fertility drugs during IVF) , Miscarriage, Egg-retrieval procedure complications, Ectopic pregnancy, Birth defects. This technique also helps in production of embryos for experimental purposes in agricultural research. During 1980 the embryos were

bisected in order to provide zygotic twins (genetically identical in terms of both their nuclear and mitochondrial genes). These are then placed in an empty zona before being transferred to different recipient mothers to carry them to term.

12.4.1DOLLY, EMBRYO TRANSFER IN HUMAN

DOLLY

The female Finn Dorset sheep named Dolly is the first clone (February 1997) of an adult mammal, produced by British developmental biologist Ian Wilmut and colleagues of the Roslin Institute, near Edinburgh, Scotland. The clones had been produced earlier in the laboratory, but they are derived from embryonic cells which are either undifferentiated or only partially differentiated. The clone of lower species like frogs has been produced previously from fully differentiated (adult) cells (e.g., skin or muscle cells).

The clone Dolly is produced from the mammary gland cell taken from an adult Finn Dorset ewe. Wilmut et al produce Dolly by using electrical pulses to fuse the mammary cell with an unfertilized egg cell, the nucleus of which had been removed. The fusion procedure caused the transfer of the mammary cell nucleus into the egg cell, which then began to divide. Derived from mammary cell nuclei of Finn Dorset ewe and host egg cytoplasm from Scottish Blackface ewes, a number of fused couplets successfully formed embryos. Then the embryos were transferred to surrogate Scottish Blackface ewes. Out of 13 recipient ewes, one became pregnant, and after 148 days of gestation period, Dolly was born. Dolly remained alive and well long after her birth and her body organs function properly. The technique which produces Dolly is now known as somatic cell nuclear transfer (SCNT). This technique is now used to produce a variety of mammalian clones from different types of adult cells. Dolly suffered from progressive lung disease and on February 14, 2003 she was euthanized by veterinarians. Her body was preserved and displayed at the National Museum of Scotland in Edinburgh.

HUMAN EMBRYO TRANSFER

Embryo transfer is a process of transplantation of a mammalian preimplantation embryo into the reproductive tract of a recipient female so that it may implant and continue to develop to birth. Mammalian embryos of many species are able to develop in vitro from fertilization to the blastocyst stage, but at this stage, they must be implanted in the uterus in order for embryogenesis to proceed normally. The first successful embryo transfer was performed in

1890 in the rabbit. Since then various improvements have been done to improve this technique. In 1978, the first birth of a human occurred from a transferred embryo. Embryo transfers are required when natural fertilization is not an option or has difficulty occurring due to ovulation disorders, damage to fallopian tubes, endometriosis, premature ovarian failure, uterine fibroids, genetic disorders, impaired sperm production, etc.

Embryo Transfer is of various types:

1) Fresh embryo transfer: The fertilized eggs are cultured for 1-2 days. The best embryos are chosen to transfer directly to the woman's uterus.
2) Frozen embryo transfer: Any healthy embryos that were not used in the first transfer can be frozen and stored for future use. These can be thawed and transferred to the uterus.
3) Blastocyst embryo transfer: If many healthy embryos develop after fertilization, it is common to wait to see if the embryos develop into blastocysts. And then transfer to the woman's uterus.
4) Assisted hatching (AH) before Transfer: Assisted hatching is done in the laboratory just before embryos are transferred into the woman's uterus. Using a laser, the embryologist makes a tiny, precise opening in the shell of each embryo after which it is transferred.

Process of Embryo Transfer

1) An embryo transfer is the last step of the in vitro fertilization (IVF) process.
2) During IVF, the ovaries are stimulated to release eggs by using fertility medications
3) These eggs are then removed from a woman's ovaries and fertilized in a lab.
4) After the fertilized eggs have multiplied, the best embryo is chosen and transferred to the womb by placing it in a soft catheter and injected in the uterine cavity through the cervix.
5) Pregnancy occurs when the embryo attaches itself to the wall uterus (implantation).

The complication associated with embryo transfer are increased hormonal stimulation (resulting in increased risk of blood clot blocking a blood vessel), bleeding, changes in vaginal discharge, infections, and complications of anesthesia if it is used. The greatest risk of embryo transfer is the chance of multiple pregnancies results due to multiple embryos attachment to the uterus. This may increase the risk of stillbirth and children born with disabilities.

Importance of Embryo Transfer Technique

- Embryo transfer helps in experiments involving removal of the developing embryo from the reproductive tract of one female animal and transfer to another.
- This technique helps in production of transgenic animals, knockout mice, and pathogen-free colonies.
- This technique helps in experiments involving separation of maternal and fetal genetic effects.

10.5 TRANSGENIC ANIMAL

Transgenic animals are created by deliberately inserting a gene into the genome of an animal. Numerous approaches are nowadays used for genetic engineering of various animal species. Various methods are used to access the germline of animals: (1) direct manipulation of the fertilized egg and then its implantation into the uterus; (2) manipulation of the sperm (3) manipulation of early embryonic tissue (4) the use of embryonic stem (ES) cell lines which, after manipulation and selection ex vivo, can then be introduced into early embryos and (5) manipulation of cultured somatic cells, whose nuclei is fused with enucleated oocytes to produce a whole animal (e.g. Dolly).

Generally two methods are used for inserting DNA into vertebrate germline cells, transfection and infection with retrovirus vectors. A third method based on the use of mobile genetic elements, has been commonly used for insects.

Transfection

Transfection process involve: (1) direct microinjection of DNA into the cell nucleus; (2) electroporation— method of DNA introduction through transient pores created by controlled electrical pulses; (3) use of polycations to neutralize charges on DNA and the cell surface that prevent efficient uptake of DNA; (4) lipofection, or enclosure of DNA in lipid vesicles that enter a cell by membrane fusion much in the manner of a virus, and (5) sperm-mediated transfection, possibly in conjunction with intracytoplasmic sperm injection (ICSI) or electroporation. The DNA structure introduced into a cell by any of the above methods is highly variable and uncertain. Generally, only a fragment of the transfected DNA is integrated into the chromosome, frequently in multiple copies, that often are integrated in long tandem arrays.

Retrovirus Vectors

Retroviruses are a class of viruses which replicate by a specific process involving copying of the viral RNA genome into DNA (reverse transcription) followed by its specific and stable introduction into host cell DNA (integration). Then the host transcriptional machinery is used to express the integrated DNA. Retroviruses are used to introduce genes of interest into cells in culture or into somatic tissue in experimental animals. They are also used for germline modification of fish, mollusks, chickens, mice and cattle. Retrovirus vectors are prepared using DNA constructs containing the gene of interest lined by sequences necessary for replication as a virus. These sequences include transcriptional promoters in the long terminal repeats (LTR's), which flank the integrated DNA, or provirus. Other necessary sequences are Signals necessary for packaging of the transcript in virions (virus particles), for reverse transcription, and for integration of the resulting DNA. These DNA constructs are introduced into cells which express viral proteins, but that are unable to make infectious viruses, leading to the creation of infectious virions containing an RNA copy of the gene of interest. When cells are infected with such virions, the RNA is copied into DNA and integrated at random sites in the cell genome. To select cells containing the desired virus construct selectable markers are included in the construct.

Transposons

Transposons are DNA elements that can transfer their information from one site to another in the same cell. A variety of transposons have been found in insects and fish and some are used as vectors for the generation of transgenic insects.

Directed genetic manipulation

Another goal of transgenic technology is to produce engineered animals that lack specific genes (knockout), or have these genes replaced by one that has been engineered in a specific way (knockin). For example, transplantation of organs or tissues from non-primates (pigs) to humans (xenotransplantation) is currently impossible, due to immune response by human recipients to a carbohydrate on the surface of pig cells (galactose-1,3-galactose). Inactivation of the enzyme (galactosyl transferase, GT) in donor pigs could solve this problem.

From the 1990s, attempts have been made to yield transgenic animals that synthesize a variety of human proteins. The coagulation factors VII, VIII and IX are used for lifelong treatment of hereditary diseases and can be produced in the milk of transgenic animals. An

immune response to therapeutic agents develops in most patients over time, despite the highly efficient purification of the proteins produced in bacterial or yeast systems. Thus production of them by transgenic animals is very useful. The demand of monoclonal Antibodies (mAb) is high in the pharmaceutical industry and they are produced exclusively in mammalian cell cultures, since proper post-translational modifications are required to ensure therapeutic efficiency. So, transgenic animals used for the production of mAb will be very useful.

10.6 *HUMAN GENE THERAPY, CRYOBIOLOGY*

HUMAN GENE THERAPY

Gene therapy is a procedure in which an effective gene is inserted into a human cell to correct a genetic error or to introduce a new function to the cell. Gene therapy has become a reality due to advancement in the field of bioengineering that helps in manipulating vectors for delivery of extrachromosomal material to target cells. Numerous approaches, including retroviral vectors and non-viral vectors, have been developed for both ex vivo and in vivo transfer of genes into the cells. The main objective of gene therapy is optimization of delivery vehicles (vectors) that are mostly plasmids, nanostructured or viruses. The viruses are used as a vector due to their quality to invading cells and inserting their genetic material into the host genome. But the problem with viral vectors is that it exacerbates immune responses and genome manipulation, especially in germ line cells. Gene therapy is a very complex process and requires new developments. It required identification and access to specific cells that needed to be treated. The method should efficiently distribute the gene copies to the cells. The diseases and their strict genetic bonds need to be totally known. The gene therapy is of two type 1) gene therapy of the germline and 2) gene therapy of somatic cells.

In germline gene therapy, the stem cells (sperm and egg) are altered by the introduction of functional genes, which are integrated into their genome. The germline modifications are hereditary and pass on to subsequent generations. Somatic cell gene therapy involves integration of therapeutic genes into the patient's somatic cells. Any change and any effects are limited only to that patient and are not inherited by future generations. Gene therapy involves the insertion of a normal gene into the genome to replace an abnormal gene responsible for producing a certain disease. To release the gene into the stem cell the molecular carrier called a "vector" is used. The vectors must be very specific, show efficacy in the release of one or more genes of the sizes required for clinical applications, not be acknowledged by the immune system, and can be purified in large amounts. After the it is

inserted into the patient, it cannot induce an immune response; it should increase the normal functions, correct deficiencies, or inhibit deleterious activities. The vector should be safe for the environment, patients and for the professionals who manipulate it. Lastly, the vector should be able to express the gene, for the patient's entire life. There are numerous safety and ethical issues concerning manipulation of the human genome that need to be resolved. Gene therapy has potential for the effective treatment of genetic disorders.

CRYOBIOLOGY

Cryobiology is the branch of science that deals with the effects of freezing and low temperatures on living organisms". Various cryobiological techniques are used today e.g. cryosurgery, embryo and gamete preservation, tissue preservation and transplantation, blood and blood product preservation, and cryotransport. Cryosurgery is used for the treatment of many types of skin lesions (including cancer), for benign and dysplastic mucosal lesions, for uterine cervicitis and intraepithelial neoplasia, and for cardiac surgery in the treatment of tachyarrhythmias. In this technique selective necrosis of tissues is done through freezing at very low temperatures. The low temperature is achieved by the use of a specific solution called cryogen. Cryosurgical procedures also involve the use of a dipstick apparatus, which is simply a cotton-tipped applicator. The dipstick is dipped into liquid nitrogen and applied to a lesion until adequate necrosis is achieved. Other methods involve the use of open-spray apparatus which help in spraying cryogen on the lesion and solidified carbon dioxide which can be directly applied to the skin with a mix of acetone to treat certain types of acne (slush therapy). Another technique used is cryoprobe in which a precooled metal accessory is applied directly to the lesion.

Cryosurgery has both advantages and disadvantages. The advantages of cryosurgery over other procedures are 1) fit for nursing home, or outpatient facility, 2) no need of general anesthesia, for local anesthesia is optional, 3) no operative suits required, the procedure is simple and safe, 4) no physical restrictions afterward and 5) availability for pregnant patients.

B. Rubinsky, et. al., of the University of California-Berkeley (1990) show negative impact of cryosurgery. They show that cryosurgery done on the liver results in formation of ice in the hepatic sinusoids, expands the sinusoids, dehydrates the hepatocytes, and causes structural damage.

Embryo and gamete cryopreservation

Cryopreservation technique employed since 1972 firstly used in case of mice then for goat (1976) and later for humans (1983). This technique results in successful birth of live young ones from frozen embryos. Cryopreservation techniques involve a number of steps which are specific and straightforward methods of freezing and are exclusive for each organism. The first step involves the exposure of the embryo to cryoprotective solution and then the embryo is cooled to slightly lower than zero degrees Celsius, followed by a seeding procedure. Seeding is a technique utilized for induction of ice formation under controlled conditions. After that, under controlled conditions of cooling the embryo is subjected to intermediate subzero temperature. The ideal temperature for cryopreservation is temperatures as low as -80 to -100 degrees Celsius and temperatures as high as -20 to -40 degrees Celsius. For long-term storage the freezing is done very rapidly to -196 degrees Celsius. If the cryopreservation is done properly the embryo can survive for thousands of years. Whenever the cryopreserved embryos are used they should thaw properly. The warming and thawing depends on the amount of freezing that had initially been done. After that the cryoprotective solution is removed otherwise improper handling of this solution is toxic to the embryo. The cryoprotective solution is like body fluids of the womb, containing sodium ion- rich saline solution.

Tissue and Blood Cryopreservation

The body tissue and blood can also be preserved using cryopreservation techniques and can be reintroduced into the body whenever required. Previously transplants like born marrow relied on time to be effective, but now cells can be frozen and stored for years and still be effective. Under laboratory conditions the desired cell can be preserved for short-term in a controlled freezer that can cool at a rate of one degree per minute, or for long-term in a large, liquid nitrogen freezer at -180 to -195 degrees Celsius. Likewise blood and blood products can be stored and used whenever required. This technique can also be used for the preservation of placentas and umbilical cords, which can be used as blood and hematopoietic stem cell sources. Later on these preserved cells can be used for stem cell matching for a child, life-threatening diseases treatment, or gene therapy.

Although cryopreservation techniques are very useful, there are various reasons for its opposition. Firstly cryobiological procedure may affect the cells and the surrounding areas leading to damage. Cryosurgery can also cause damage and this damage can result in future problems that will need to be cured, generating a vicious cycle. During cryopreservation

embryos or gametes can get damaged resulting in less viable births. The damage is due to poor technique, simply inactive embryos, or damage sustained by cryoprotective solutions or the freezer. The cryopreservation technique is very expensive for the average person to afford. Legal issues are also associated with embryo preservation. For example a couple decided to preserve their embryo but in future if they get separated then the custody issue arises "Who will have the legal right to embryo?"

10.7 *SUMMARY*

Biotechnology offers new tools for improving health and productivity of organisms. It helps in detection, treatment and prevention of diseases. Biotechnology helps in various sectors of agriculture by improving overall health, breed and productivity of livestock. Cell culture refers to laboratory methods that enable the growth of cells in physiological conditions in vitro. The cell culture technique originated in the 20th century and it was used to study growth and maturation of tissue, particular gene role in disease and metabolism, for large-scale production of biopharmaceuticals by hybrid cell lines, study of viruses and vaccine development. Cells cultured can be classified into three different types: primary cells, transformed cells, and self-renewing cells. Cell culture has few limitations like it doesn't represent the in vivo responses due to lack of an appropriate micro environmental context of the responding cell types. So, organ culture provides an opportunity to better replicate the tissue microenvironment. Embryo culture is a procedure for the cultivating of an embryo under aseptic conditions on a culture medium. The aim of embryo culture technique is to improve the quality of embryos developed in the laboratory condition. The embryos are cultured after 2 to 3 days to reach the four-to-eight cell stage before transfer to the uterus. Embryo transfer is a process of transplantation of a mammalian preimplantation embryo into the reproductive tract of a recipient female so that it may implant and continue to develop to birth. In vitro fertilization (IVF) is a complex series of events in which mature eggs are collected (retrieved) from ovaries and fertilized by sperm in a lab. The female Finn Dorset sheep named Dolly is the first clone (February 1997) of an adult mammal, produced by British developmental biologist Ian Wilmut and colleagues of the Roslin Institute, near Edinburgh, Scotland. Transgenic animals are created by deliberately inserting a gene into the genome of an animal. Gene therapy is a procedure in which an effective gene is inserted into a human cell to correct a genetic error or to introduce a new function to the cell.

Gene therapy has become a reality due to advancement in the field of bioengineering that helps in manipulating vectors for delivery of extrachromosomal material to target cells Cryobiology is the branch of science that deals with the effects of freezing and low temperatures on living organisms. Various cryobiological techniques are used today e.g. cryosurgery, embryo and gamete preservation, tissue preservation and transplantation, blood and blood product preservation, and cryotransport.

10.8TERMINAL QUESTION AND ANSWERS

1. Study of preservation of life at low temperature is

A) Malacology

B) Icebiology

C) Exobiology

D) Cryobiology

2. What does IVF stand For?

A) In vivo fertilization

B) In vitro fermentation

C) In vivo fermentation

D) In vitro fertilization

3. Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as _____

A) Transgenic animals

B) Modified animals

C) Infected animals

D) Bt animals

4. The sheep "Dolly" was cloned by using somatic cell from the donor's

A) Mammary gland cell

B) Liver

C) Kidney

D) Eye

5. Disaggregating of cell can be done using

A) Physical disruption

B) Enzymatic digestion

C) Treating with chelating agents

D) All of the above

Answers: 1(D),2(D),3(A),4(A),5(D)

Q 6. Give detail of cell culture technique.

Q 7.Write a short note on

a) Cryobiology
b) Embryo culture
c) Human gene therapy

Q 8. Explain the process of in vitro fertilization.

10.10 *REFERENCES*

1. Cell culture Growing cells as model systems In Vitro Charis, P Segeritz and Ludovic Vallier. Basic Science Methods For Clinical Researchers. 2017:151-172
2. Human Organ Culture: Updating the Approach to Bridge the Gap from In Vitro to In Vivo in Inflammation, Cancer, and Stem Cell Biology, Rafia S. Al-Lamki, John R. Bradley, and Jordan S., PoberFront Med (Lausanne). 2017; 4: 148.
3. Early Embryo Rescue Renee Drewes-Alvarez, in Reference Module in Life Sciences, J Turk, Ger Gynecol Assoc. 2011; 12(2): 110–117.
4. Embryo culture media for human IVF: which possibilities exist?, Irmhild Gruber and Matthias KleinJ Turk, Ger Gynecol Assoc. 2011; 12(2): 110–117
5. https://www.britannica.com/topic/Dolly-cloned-sheep

6. https://thebiologynotes.com/human-embryo-transfer December 30, 2019 by Yashaswi Sharma
7. Animal Biotechnology: Science-Based Concerns Washington (DC): National Academies Press (US); 2002. ISBN-10: 0-309-08439-3
8. Use of Transgenic Animals in Biotechnology: Prospects and Problems, O. G. Maksimenko, A.V. Deykin, Yu. M. Khodarovich, and P. G. Georgiev, Acta Naturae. 2013 Jan-Mar; 5(1): 33–46
9. Gene therapy: advances, challenges and perspectives, Giulliana Augusta Rangel Gonçalves and Raquel de Melo Alves Paiva,Einstein (Sao Paulo). 2017 Jul-Sep; 15(3): 369–375
10. Cryobiology: Low temperature studies of biological systems By Krista Nussbaum https://www.goshen.edu/bio/Biol410/BSSpapers99/krista/Krista.htm

UNIT 1: IMMUNOLOGY EXPERIMENT/STUDY

1.1 DLC (Differentiation Leucocytes count)

A white blood cell (WBC) count measures the number of white blood cells in our blood, and a WBC differential determines the percentage of each type of white blood cell present in our blood. A WBC count can also be called a leukocyte count, and a WBC differential can also be called a leukocyte differential count.

Blood (A.S., *blod,* blood) is defined as a specialized connective tissue which is an opaque, thick, rather viscid (viscosity of blood ranges from 4.5 to 5.5) liquid of red colour, salty to taste (contains about 0.85-0.90% NaCl) and alkaline (pH 7.4) in reaction. It flows through the circulatory system, helps in the transport of nutrients, oxygen (O_2), carbon dioxide (CO_2), waste products (metabolic), hormones, etc. and performs different important physiological functions including immunity. Blood is composed of liquid intercellular substance known as **plasma** (Gk. *Plasma,* form) and formed elements such as **erythrocytes** Gk. *Erythros,* ed; *kytos,* hollow(RBC), **leucocytes** Gk. *Leukos*, white; *kytos,* hollowWBC and **platelets** (F. *plat*, Gk. *Platys,* flat) that remain suspended in the plasma.

A mature human red blood corpuscle (RBC) or erythrocytes is defined as a formed element which is a circular, biconcave and non-nucleated cell which has haemoglobin in its cytoplasm, and helps in the transport of O_2 and CO_2.

In adults, red bone marrow proerythrobloast cells or **proerythrocytes** (Gk. *Pro,* before; *erythros,* red; *blastos,* bud) is the site of origin of RBCs (erythropoiesis). The cell membrane of a red blood cell becomes fragile and the cells are rendered non-functional in about 120 days. Under normal circumstances, the average count of RBCs in the peripheral blood of male adults is around 5 million per cubic millimetre of blood. In adult females, however, it is around 4.5 million. Different clinical conditions (anaemia, polycythaemia, etc.) and physiological states (time of the day, muscular exercise, attitude etc.) are responsible for the variation and deviation from the normal RBC count of an individual. Average life span of an RBC is about 120 days in the blood. Destruction and disintegration of old and senile RBCs takes place in the **reticulo-endothelial** (L. *reticulum*, small net; Gk. *Endon*, within; *thele,*

nipple) cells of spleen and liver. To maintain normal quantities of erythrocytes, the body produces new mature RBCs at the astonishing rate of 2 million/second.

White blood corpuscles or leucocytes are defined as formed elements of blood which are nearly circular in shape, larger than RBCs, possess indefinite sized nucleus, carries no haemoglobin like pigment in its granulated or agranulated cytoplasm and helps in the defence mechanisms (immunity) of our body.

While granulocytic WBCs originate **(leucopoiesis)** from **myeloblast**or **promyelocytes** of the bone narrow, agranulocytic WBCs such as **lymphocytes** and **monocytes** originate mainly from **lymphoblast** and **monoblast** cells of **lymph nodes.** The average total number of WBC is 6000-8000/cubic-millimeter (range 5000-10000). The average ratio of WBC

Haematological values in percent								
Study items	**Male**				**Female**			
	6 weeks		**10-12 weeks**		**6 weeks**		**10-12 weeks**	
Complete blood count (CBC)	WBC	6.80	WBC	5.24	WBC	4.80	WBC	6.71
	RBC	7.10	RBC	7.82	RBC	6.90	RBC	8.10
	Hb	15.50	Hb	16.40	Hb	14.60	Hb	18.63
Differential Count (DC)	N	8.20	N	11.33	N	9.70	N	13.88
	L	91.60	L	88.11	L	89.80	L	85.75
	M	00.20	M	00.44	M	00.40	M	00.25
	E	00.00	E	00.11	E	00.10	E	00.13
	B	00.00	B	00.00	B	00.00	B	00.00

N = Neutrophil, L = Lymphocyte, M = Monocyte, E = Eosinophil, B = Basophil

to RBC total count is 1:700. This count varies under different clinical conditions and altered physiological states. The life span of WBCs is variable, starting from 12-15 days of basophils

to 2-3 days of lymphocytes. Old and senile WBCs undergo destruction and are removed from the system by different mechanisms.

To examine the total count of RBC and/or WBC, a definite volume of blood is diluted with a known amount of diluents and the number of corpuscles is counted in the known volume of this mixture under microscope with the help of an instrument called **haemocytometer.** The date gathered by this method is used for enumerating the total number of corpuscles in the sample. However, such enumeration is done mathematically.

AREAS OF APPLICATION

In normal human beings, total leucocytes' count range from 5,000 to 10,000 per µl of blood. A count below this range, known as leukopenia, can be observed in case of heavy metal poisoning, radiation sickness or infections (measles, mumps, chicken pox, poliomyelitis, influenza, typhoid fever, and AIDS). It can also be produced by glucocorticoids, anti-cancer drugs and immunosuppressant drugs.

A leucocytes' count on the contrary, above 10.000 white blood cells per µl of blood, called exocytosys usually indicates infection, allergy, varied diseases, dehydration and emotional disturbances. Since different types of leucocytes increase or decrease in response to an enormous variety of ailments, given below is a short tabular representation that describes normal and abnormal distribution of leucocytes with possible correlation of the type of ailments that the leucocytes respond to.

Total 4.3b Normal and abnormal distribution of leucocytes

Name of leucocytes	Percentage in normal subjects	Clinical applications vis-à-vis functional aspects

Neutrophils (_____ span 7 hours in blood)	60%-70-%; Mean count: 4150 cells/µl of blood	Involved in immune defence. Increases in response to bacterial infection.
Eosinophil's (life span: unknown)	2%-4% Mean count: 165 cells/µl of blood	Increases in parasitic infections, allergies, collagen associated diseases, and the diseases of spleen and central nervous system.
Basophils (life span: unknown)	<0.5%-1%; mean count: 44 cells/µl of blood	Involved in inflammatory responses. Relatively stable, although elevated during allergic reactions.
Lymphocytes (life span: unknown)	25%-33%; mean count: 2185 cells/µl of blood	Involved in antibody production and cellular immune responses. Increases in diverse infections and immune responses.
Monocytes (life span: 3 days in blood)	3%-8%; Mean count: 456 cells/µl of blood	Involved in immune surveillance. Increases in viral infection, and inflammations. Elevated count of monocytes generally indicates chronic infections.

THEORETICAL FUNDAMENTALS

Differential count involves determination of the number of each kind of white blood cells (collectively called leucocytes) in a sample of 100 cells for clinical diagnostic purposes. Altogether, there are five different kinds of leucocytes in man-**neutrophils, eosinophils, basophils, lymphocytes,** and **monocytes.** The first three are considered to be the members of a group, named as **granulocytes,** while the rest two belong to another group, known as **granulocytes.**

Three important criteria are considered most useful for the purpose of identifying the leucocytes.

1. Cytoplasmic granules
2. Cell size
3. Nuclear shapes of the leucocytes

All these characteristics for each kind of leucocytes are presented in Table 4.3.

EXPERIMENTAL METHODOLOGY

Materials required

(i) Glass slides, (ii) 70% alcohol, (iii) sterilized needles, (iv) Leishman's stain, (v) double distilled water, (vi) Pasteur pipette and (vii) compound microscope.

Table 4.3 Identifying characters and other relevant information's with respect to white blood cells in man

	Diameter (μm)	Cytoplasmic granules	Nuclear shape	Specialities
Neutrophils	09-12	Fine granules (violet)	Divided into 3 to 4 lobes with fine strands of cytoplasm.	Young cells sometimes possess undivided nucleus.

Eosinophil's	10-14	Course granules (rosy)	Bilobed nucleus.	Bilobed nucleus appears as if two ballons are tied together
Basophils	08-10	Course granules (purple	S or U-shaped nucleus.	Nucleus is made obscure from view by course granules in cytoplasm.
Lymphocytes	5-8 (small) 10-12 (medium) 14-17 (large)	-	Large nucleus; almost fills the cell leaving only a narrow rim of cytoplasm.	Many intermediate forms are possible; light microscopic study appears difficult
Monocytes	12-15	-	Round/oval/lobed/kidney shaped/C-shaped; cytoplasm is abundant and clear	Largest of all leucocytes; usually twice the size of erythrocyte.

4. Gently mix the bacteria, and spread the bacteria over the slide covering an area not more than 1 cm^2.

Fixation

For fixation (and also drying of cells), hold the slide horizontally with forceps and quickly pass it just over the yellow flame of Bunsen burner two to three times. This step is to be done (For beginners) under direct supervision of your instructor, because overheating may spoil

the entire endeavour. Fixing cells in such a way kills the bacteria (by coagulating the cytoplasm) and makes them stick to the slide.

Staining

1. Flood the slide with 0.5% crystal violet stain, and leave for about 30 seconds. Then pour off the excess stain (Crystal violet makes all bacteria violet).
2. Flood the slide with Lugol's iodine, leave it for 30 seconds and then wash off the iodine with distilled water (iodine fixes the stain more permanently onto the cells).
3. Wash with acetone-alcohol for not more than 2-5 seconds. Immediately wash with water to prevent excessive decolourization
4. Counterstain with freshly filtered neutral red for 1-2 minutes/safranin for 1 minute. Counter staining imparts red colour on the Gram-negative bacteria.
5. Wash with water and blot dry (hold the slide between sheets of clean blotting paper, and finally allow drying in air).

OBSERVATIONS

Examine the slide under oil immersion lens of a good quality compound microscope. The results will ideally be as follows:

1. Gram-positive organisms (bacteria) will appear violet or blue-black (e.g., *Lactobacillus* sp.). Gram-negative bacteria will appear red (e.g., *Escherichia coli*, *Azotobacter* sp.).

AREAS OF APPLICATION

Gram staining technique is considered the most basic tool in classifying bacteria as well as in different pathological diagnostics.

Materials required

(i) Improved Neubauer haemocytometer, (ii) a thick square cover glass, (iii) two pipettes for drawing of blood-RBC pipette and WBC pipette, (iv) RBC diluting fluid, (v) WBC diluting

fluid, (vi) lancet or needle, (vii) cotton (non-absorbent), (viii) rectified spirit (90% ethanol) and (ix) compound microscope with halogen light source.

Description of the apparatus

Haemocytometer

The improved Neubauer haemocytometer is a thick glass slide on which there are three flat platforms extending across the slide. These platforms are separated from each other by deep grooves. The middle platform is 0.1 mm lower than the other two platforms. On the surface of the middle platform there are two sets (duplicate) of ruled area. The area consists of 9 large squares, each having 1 square millimetre area and is elaborately ruled. The four corner squares are divided into 16 equal sized squares. The central square is ruled into 25 groups of 16 small squares, each group is separated by triple lines. The ruled surface is 1/10 mm below from the inner surface of the cover glass placed over the-middle platform, so each smallest square of the corner has the volume of ¼ X ¼ X 1/10 or 1/160 cubic millimetre, while the smallest square of the canter has the volume of 1/20 X 1/20 X 1/10 or 1/4000 cubic millimetre.

RBC pipette

It bears three graduations-0.5 and 1 is on the stem of the pipette, and the 101 mark is placed just above the bulb of the pipette. There is a red bead kept inside the bulb. The pipette is fitted to a rubber tube.

WBC pipette

This is similar to the RBC pipette in structure except for the graduations which are 0.5, 1 and 11 instead. There is one white bead kept inside the bulb.

Preparation of solutions/reagents

RBC diluting fluid

It is an isotonic solution which prevents coagulation, haemolysis, rouleaux formation, and bacterial growth. Hayem's diluting fluid contains the following:

Sodium sulphate (Na_2SO_4) - 5.0 g

Sodium chloride (NaCl)	-	1.0 g
Mercuric chloride ($HgCl_2$)	-	5.0 g

In distilled water (final volume: 200 ml)

WBC diluting fluid

It contains a weak acid (to lyses RBCs) and a dye (for staining the nucleus of white blood cells) in the following composition-Glacial acetic acid (1.5 to 3 ml) and distilled water (96.5 to 97 ml). To the acetic acid solution of this specified strength, a few drops of an aqueous solution of methylene blue or gentian violet are added for obtaining WBC diluting solution.

Total count of RBCs

1. Puncture the fingertip of volunteers/patients/subjects aseptically by a lancet or needle. Wipe off the first drop of blood by cotton and allow the blood to flow freely.
2. Draw blood into the RBC pipette up to 0.5 or 1 mark.
3. Wipe off extra blood from the outer surface of the pipette and immediately draw RBC fluid immediately up to 101 marks.
4. Mix the contents of the bulb thoroughly by using the red coloured bead present inside the bulb of the pipette. Rotate the pipette well for about a minute holding in a horizontal position and finally shake sidewise; perform mixing carefully and avoid any leakage).
5. When blood is drawn up to 1 mark then dilution becomes 100 fold, but when blood is drawn up to 0.5 then 200 fold VC dilutions is achieved (because last part of the fluid remains locked up in the stem and is not available for dilution).
6. Place the cover glass properly in position over the ruled area of haemocytometer.
7. Expel the clear fluid from the stem of the pipette which does not contain blood. Quickly put a drop of diluted blood at the edge of cover glass placed over the counting chamber, and allow the very drop to flow under the cover glass by capillary action. Overflow of fluid or introduction of air bubble inside the chamber should strictly be avoided.

8. Allow the cells to settle at the bottom of the chamber for 2-3 minutes, but avoid drying up of the material, (For this take a petridish, place a wet cotton or filter paper at its bottom and then place the charged counting chamber on to it. Finally cover up the dish and keep in this condition for about 2-3 minutes.)

9. Place the haemocytometer on the stage of the microscope. Focus the light and reduce its intensity by lowering the condenser and partially closing the diaphragm (examine under 40X or 20X objective lens of the microscope).

10. Count the RBCs at the five groups of 16 smallest squares of the central square in ruled area. Stated otherwise, count the number of RBC present in 80 smallest squares.

11. When properly focused, the red corpuscles are sharply defined and the rulings appear as well-defined black lines.

12. Calculate the total count of RBC using the following procedure:

    If the number of RBC present in 80 smallest squares = *x*,

    Then one smallest square contains *x*/80 RBCs, or 1/4000 cubic millimeter of the diluted blood contains *x*/80 RBCs.

    Hence, one cubic millimeter of undiluted peripheral blood of the individual (volunteer/patient/subject), contains *x*/80 x 4000 x dilution = Z of RBCs.

Total count of WBCs

1. Following the same procedure (as described for TC of RBC) draw blood into the WBC pipette up to 0.5 or 1 mark, and then quickly draw WBC diluting fluid up to 11 mark.

2. Following the procedure described in steps 6-10 with respect to RBCs' count the number of WBCs under 10X objective lens of the microscope.

3. Count the WBCs present in the four corner squares of the ruled area. Each of these four squares is subdivided into 16 smaller squares. Stated otherwise, count the number of WBCs present in (16 x 4) or 64 small squares.

4. Calculate the total count of WBCs using the following procedure:

   If the number of WBC in 64 small squares = *x*,

   Then one small square contains *x*/64 WBCs, or 1/160 cubic millimetre of the diluted blood contains *x*/64 WBCs.

   Hence, one cubic millimetre of undiluted peripheral blood of the individual (volunteer/patient/subject), contains *x*/64 x 160 x dilution = Y number of WBCs.

AREAS OF APPLICATION

Total count of RBCs and WBCs is included in the list of routine tests in most of the clinical pathology laboratories. Results of these tests are used for differential diagnosis of different clinical conditions.

EXPERIMENTAL METHODOLOGY

Materials required

(i) Aqueous solution (0.5%) of crystal violet, (ii) Lugol's iodine (3 g iodine and 6 g potassium iodine in 900 ml of distilled water), (iii) acetone alcohol (1:1 v/v), (iv) safranin (1% aqueous solution), (v) wire loop, (vi) Bunsen burner, (vii) glass slide (wiped with alcohol), (viii) forceps, (ix) petri dishes, (x) distilled water in wash bottle, (xi) blotting paper, (xii) compound microscope and (xiii) bacterial culture on plate.

Preparation of bacterial smear

1. Take a wire loop, flame the same on Bunsen burner and allow it to cool.
2. Take two loopful of distilled water on the centre of a clean of a clean glass slide.
3. Bring the wire loop in touch with the bacterial colony of interest (or provided) very quickly by opening the lid of the plate (for the reasons of safety against contamination) and transfer the bacteria in the loop to the glass slide.

Method/Protocol

1. Clean the slides with absolute alcohol.

2. Clean the tip of middle finger of the subject (individual) and prick with a sterilized needle.

3. Wipe away the first drop of blood that appears on the fingertip with sterilized cotton, soaked in alcohol. Take the second drop of **free flowing** blood on the clean slide about 1 cm from the right narrow edge of it.

4. Place the narrow edge of the second slide making an angle of 45^0 to the first slide just left to the drop of blood to be examined.

5. Slowly pull the second slide, holding in position, to the right until the second slide touches the blood on the first. Immediately afterwards, push the second slide to the left at uniform speed. Such leftward movement of the second slide produces a thin blood film on the first slide. Make 3-4 such blood films.

6. Place the slide with blood smear in a dust-free open space for 10 minutes to get the smear air-dried properly.

7. Put a scratch mark on the first slide on one corner on the surface containing the blood smear.

8. Put appropriate quantity of Leishman's stain on the air-dried blood smear, and leave for 5-10 minutes. After this stage put an equal amount of buffered water (composition-3.76 g $Na_2HPO_42H_2O$ + 2.10 g anhydrous KH_2PO_4 with a maximum volume of 1000 ml with double distilled water) on the dye, and leave for another 3-5 minutes. Now drain off the dye and rinse the slide with distilled water for once or twice. Finally, dry up the stained slide in air. Now the slide is ready for microscopic observation.

1.2 BLOOD GROUPS AND RH FACTORS

The ABO blood group system involves two antigens and two antibodies found in human blood. The two antigens are antigen A and antigen B. The two antibodies are antibody A and antibody B. The antigens are present on the red blood cells and the antibodies in the serum. Regarding the antigen property of the blood all human beings can be classified into 4 groups, those with antigen A (group A), those with antigen B (group B), those with both antigen A

and B (group AB) and those with neither antigen (group O). The antibodies present together with the antigens are found as follows:

1. Antigen A with antibody B
2. Antigen B with antibody A
3. Antigen AB has no antibodies
4. Antigen nil (group O) with antibody A and B.

There is an agglutination reaction between similar antigen and antibody (for example, antigen A agglutinates the antibody A and antigen B agglutinates the antibody B). Thus, transfusion can be considered safe as long as the serum of the recipient does not contain antibodies for the blood cell antigens of the donor.

The *ABO system* is the most important blood-group system in human-blood transfusion. The associated anti-A and anti-B antibodies are usually *immunoglobulin M*, abbreviated IgM, antibodies. It has been hypothesized that ABO IgM antibodies are produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses, although blood group compatibility rules are applied to newborn and infants as a matter of practice.[11] The original terminology used by Karl Landsteiner in 1901 for the classification was A/B/C; in later publications "C" became "O". Type O is often called *0* (*zero*, or *null*) in other.

Phenotype	Genotype
A	AA or AI
B	BB or BI
AB	AB
O	II

Rh blood group system

The Rh system (Rh meaning *Rhesus*) is the second most significant blood-group system in human-blood transfusion with currently 50 antigens. The most significant Rh antigen is the D antigen, because it is the most likely to provoke an immune system response of the five main Rh antigens. It is common for D-negative individuals not to have any anti-D Ig G or Ig M antibodies, because anti-D antibodies are not usually produced by sensitization against environmental substances. However, D-negative individuals can produce IgG anti-D antibodies following a sensitizing event: possibly a fetomaternal transfusion of blood from a fetus in pregnancy or occasionally a blood transfusion with D positive RBCs. Rh disease can develop in these cases. Rh negative blood types are much less common in Asian populations (0.3%) than they are in European populations (15%).The presence or absence of the Rh(D) antigen is signified by the + or − sign, so that, for example, the A− group is ABO type A and does not have the Rh (D) antigen.

1.3 WIDAL TEST FOR THE IDENTIFICATION OF TYPHOID

In 1896 and named after its inventor, Georges-Fernand Widal, is a presumptive serological test for enteric fever or undulant fever whereby bacteria causing typhoid fever is mixed with a serum containing specific antibodies obtained from an infected individual. In cases of *Salmonella* infection, it is a demonstration of the presence of O-soma false-positive result. Test results need to be interpreted carefully to account for any history of enteric fever, typhoid vaccination, and the general level of antibodies in the populations in endemic areas of the world. Typhidot is the other test used to ascertain the diagnosis of typhoid fever. As with all serological tests, the rise in antibody levels needed to perform the diagnosis takes 7–14 days, which limits its applicability in early diagnosis. Other means of diagnosing *Salmonella typhi* (and *paratyphi*) include cultures of blood, urine and faeces. These organisms produce H_2S from thiosulfate and can be identified easily on differential media such as bismuth sulfite agar.

Widal agglutination. **Widal** agglutination was introduced as a serologic technique to aid in diagnosis of **typhoid** fever. The **test** was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens ofSalmonella typhi.

The Widal test is one method that may be used to help make a presumptive diagnosis of enteric fever, also known as typhoid fever. Although the test is no longer commonly performed in the United States or other developed countries, it is still in use in many emerging nations where enteric fever is endemic and limited resources require the use of rapid, affordable testing alternatives. While the method is easy to perform, concerns remain about the reliability of the Widal test. It is not specific for typhoid fever and can be positive when a person does not have the infection.

Enteric fever is a life-threatening illness caused by infection with the bacterium *Salmonella enterica* serotype Typhi (*S. typhi*), usually transmitted through food and drinks contaminated with fecal matter. It is associated with symptoms that include high fever, fatigue, headache, abdominal pain, diarrhea or constipation, weight loss, and a rash known as "rose spots." Early diagnosis and treatment are important because serious complications, including severe intestinal bleeding or perforation, can develop within a few weeks.

The infection is rare in the U.S. and other industrialized nations but is more common in developing countries, including India, parts of South, East and Southeast Asia, and countries in Africa, the Caribbean, Central and South America, and Eastern Europe. Cases of enteric fever in the U.S. are usually attributed to travelers to these endemic areas.

In the U.S. and other developed nations, testing for enteric fever usually involves a blood culture to detect the bacteria during the first week of fever. A stool, urine or bone marrow culture may also be performed. A blood culture, however, can be labor- and time-intensive in areas of the world that lack the resources for automated equipment. In developing countries, such as those in Africa, the Widal test continues to be used instead of cultures because it is quicker, simpler, and less costly to perform.

The World Health Organization (WHO) has said that due to the various factors that can influence the results of a Widal test, it is best not to rely too much on this test. WHO instead recommends the use of cultures, whenever possible? Until another simple, inexpensive, and reliable option becomes available, however, use of the Widal test will probably persist in those countries with limited resources. There are newer rapid antibody tests for typhoid fever commercially available, several of which have been included in comparative studies of their reliability, for example in India and Africa. Findings seem to vary as to whether any are as reliable as blood culture for diagnosing this infection.

1.4 VDRL TEST FOR SYPHILIS

The venereal disease research laboratory (VDRL) test is designed to assess whether you have syphilis, a sexually transmitted infection (STI). Syphilis is caused by the bacterium *Treponema pallidum*. The bacterium infects by penetrating into the lining of the mouth or genital area.

The VDRL test doesn't look for the bacteria that cause syphilis. Instead, it checks for the antibodies your body makes in response to antigens produced by cells damaged by the bacteria. Antibodies are a type of protein produced by your immune system to fight off invaders like bacteria or toxins. we don't need to have the symptoms of syphilis for this test to be accurate. Because it checks for antibodies produced as a result of a syphilis infection, the VDRL test can be used regardless of whether you currently have any symptoms.

Early symptoms that may prompt your doctor to order this test include:

- one small, painless sore
- swelling in lymph nodes near the sore
- a skin rash that doesn't itch

In other cases, doctor may screen for syphilis even if patient don't have any symptoms. For example, doctor will screen for syphilis as a routine part of your care if you're pregnant. This is a standard procedure, and it doesn't mean your doctor thinks you have syphilis.

If patient being treated for another STI such as gonorrhea, if patient infected with HIV, or if patient engaged in high-risk sexual activity. If patient already been treated for syphilis, the Centers for Disease Control and Prevention (CDC) Trusted Source recommend follow-up testing to be sure that the treatment worked and the infection has been cured.

Usually, all we need to do for the VDRL test is allow a healthcare professional to draw our blood. Blood is generally drawn from a vein at the crease of the elbow or the back of the hand. This blood sample will then be sent to a laboratory and tested for the antibodies produced as a result of syphilis.

The VDRL test doesn't require you to fast or stop taking any medications. If doctor wants you to make an exception, they'll let you know before your test. If your doctor suspects that the syphilis infection has spread to your brain, doctor may choose to test our spinal fluid in addition to our blood.

1.5 REFERENCE

- U.S. National Library of medicine (Medline Plus**).**
- https://www.healthline.com/health/vdrl-test#procedure.
- Some important and latest point taken from Google website.
- Rana S.V.S,Biotechniques,Theory and Practices

UNIT 2: MICROBIOLOGY

2.1 PREPARATION OF CULTURE MEDIA, STERILIZATION

Bacteria (Gk. *Bacterion,* little

rod) are the most primitive and simplest organisms known. They are included under the Kingdom Monera in the five Kingdom system of classification (Whittaker, 1959). Although about 2,500 species of bacteria have so far been described and identified (and have been given names), there exist a huge body of unidentified species of bacterial Most bacterial species are single-celled organism, although a few are multicellular and filamentous. Bacteria appear as spherical, rod-shaped, or spiral-shaped organisms. Bacteria are broadly classified as **eubacteria** and **archaebacteria**. Eubacteria include those that are commonly found in living organisms, soil and water. The bacterial species belonging to Archaebacteria are, however, found in more hostile surroundings like hot springs, salty marshes, methane rich marshes, or in the ocean depths.

Bacteria vary greatly in size. The mean diameter can range from as small as 100 mm to as long as 10 µm. The extreme variation in size is reflected by the fact that there exists a bacterium, *Epulopiscium fishelsoni* by name that is a million times larger than *Escherichia coli*-the rod-shaped bacterium that is extensively used in the field of molecular biology, gene technology, molecular genetics and microbial pathology. Most eubacteria are encased by a strong cell wall (a general term that refers to the rigid, outermost layer of cells of plants, some protists, and most bacteria; the cell wall surrounds the cell membrane/plasma membrane), in which a carbohydrate matrix is cross-linked by short polypeptide chains. The characteristic composition of cell wall is often used to distinguish bacteria into Gram-positive and Gram-negative categories depending on their ability to retain or not to retain a stain, called Gram stain. The name ‘Gram’ refers to the Danish microbiologist and physician Hans Christian Gram (1853-1938), who developed a staining procedure in 1884, to distinguish two classes of bacteria as a way to detect the presence of certain disease-causing bacteria. In

general, Gram-positive bacteria are those that possess a single, thick cell wall that is able to retain the Gram stain. In the case of Gram-negative bacteria, however, the cell wall is thinner which is unable to retain Gram-stain, when subjected to it.

PREPARATION OF MEDIA AND CULTURE

CULTURE MEDIA

The method for the preparation of basic microbiology media is given below. In situations where preparation is uneconomic in time, prepared, sterilized media (liquid and solid) are available from the major school science equipment suppliers. Sterilization is at 121 °C (15 lb in $^{-2}$) for 15 minutes. pH values are 7.0 unless stated otherwise.

Note: Allow 15 cm^3 of agar for each Petri dish and 5-10 cm^3 of broth for each McCartney bottle. All cotton wool plugs should be made of non-absorbent cotton wool. Plastic or metal caps may also be used.

NUTRIENT AGAR

Suspend 28 g of nutrient agar powder in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense as required and sterilize.

NUTRIENT BROTH

Add 13 g of nutrient broth powder to 1 litre of distilled water. Mix well. Dispense as required and sterilize.

MALT EXTRACT AGAR

Suspend 18g agar powder in 1 litre of distilled water. Bring to the boil to dissolve completely. Add 15g malt extract per litre. Mix well. Dispense as required and sterilize.

MANNITOL YEAST EXTRACT AGAR

Suspend 10 g agar in 1 litre of distilled water. Heat to dissolve. Add 0.5 g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.2 g $CaCl_2.6H_2O$, 10 g mannitol and 0.4 g yeast extract. Dispense as required and sterilize.

GLUCOSE NUTRIENT BROTH

Make up nutrient broth as already directed and add 10 g per liter of glucose.

SUGAR PEPTONE WATER

Add 10 g of peptone, 5 g of NaCl, 5 g of sugar and 20 cm^3 of Universal indicator to 1 litre of distilled water; pH should be 7.4. Dispense as required and sterilize.

TRIBUTYRIN AGAR

Supplied ready for use. Heat to melt and dispense aseptically. May be prepared by adding 1% tributyrin to nutrient agar.

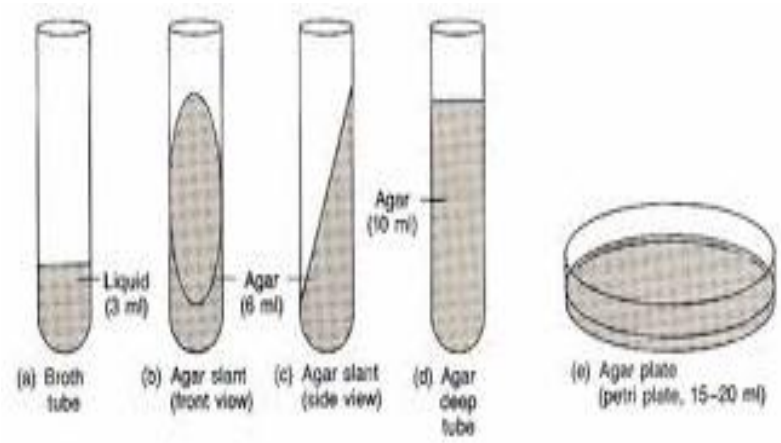


Fig .Preparation of Culture Media

GLUCOSE YEAST EXTRACT BROTH

Add 10 g of peptone, 5 g of NaCl, and 3 g of yeast extract to 1 liter of distilled water. Dispense as required and sterilize.

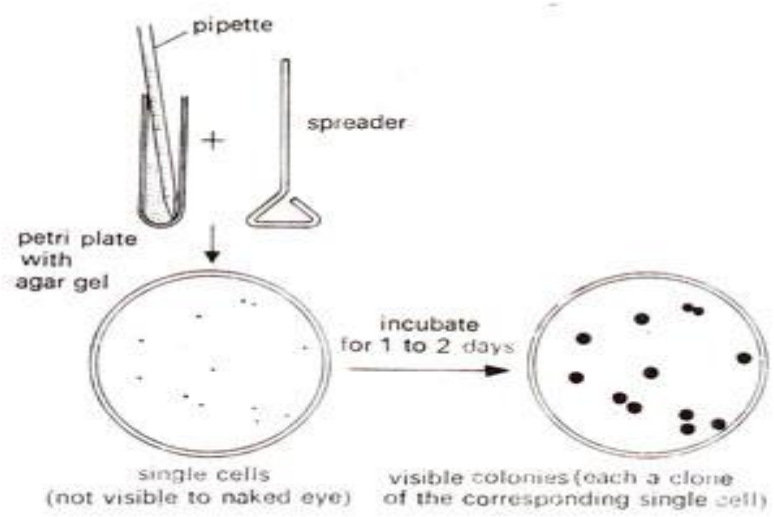


Fig: Culture media and Mutant Strains

GLUCOSE YEAST EXTRACT LEMCO BROTH

Add 10 g of Lemco (meat extract) to glucose yeast extract broth.

MILK AGAR

Make up nutrient agar as above but using only 900 cm^3 of distilled water. Dissolve 20 g of dried skimmed milk in 100 cm^3 of distilled water. Sterilize separately. Transfer the milk to the agar aseptically after cooling to 45-50 °C. Dispense aseptically.

STARCH AGAR

Suspend 15 g of nutrient agar in 100 cm^3 distilled water. Bring to the boil to dissolve completely. Heat 40 g of soluble starch in 100 cm^3 of distilled water to form a suspension. Allow to cool and then mix with the nutrient agar solution. Dispense and sterilize.

IODINE SOLUTION

Dissolve 1 g of iodine crystals and 2 g of potassium iodine in 300 cm^3 of distilled water.

Cellulose broth (for *Trichoderma reesei*)

- 800 cm^3 distilled water
- 0.1 g $CaCl_2$
- 0.5 g $(NH_4)_2SO_4$
- 0.5 g yeast extract powder
- 0.5 g asparagines
- 10 g carboxymethylcellulose
- 1.0 g KH_2PO_4
- pH6.2

Mix ingredients, heat gently, and stir until dissolved.

2.2 GRAM STAINING

Gram stain or **Gram staining**, also called **Gram's method**, is a method of staining used to distinguish and classify bacterial species into two large groups (Gram-positive and Gram-negative). The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out. They are stained pink by the counterstain, commonly safranin or fuchsine.

The Gram stain is almost always the first step in the preliminary identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. This gives rise to *Gram-variable* and *Gram-indeterminate* groups.

Gram staining is a bacteriological laboratory technique used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls. Gram staining is not used to classify archaea, formerly archaeabacteria, since these microorganisms yield widely varying responses that do not follow their phylogenetic groups.

The Gram stain is not an infallible tool for diagnosis, identification, or phylogeny, and it is of extremely limited use in environmental microbiology. It is used mainly to make a preliminary morphologic identification or to establish that there are significant numbers of bacteria in a clinical specimen. It cannot identify bacteria to the species level, and for most medical conditions, it should not be used as the sole method of bacterial identification. In clinical microbiology laboratories, it is used in combination with other traditional and molecular techniques to identify bacteria. Some organisms are Gram-variable (meaning they may stain either negative or positive); some are not stained with either dye used in the Gram technique and are not seen. In a modern environmental or molecular microbiology lab, most identification is done using genetic sequences and other molecular techniques, which are far more specific and informative than differential staining.

STAINING MECHANISM

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas Gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. There are four basic steps of the Gram stain:

- Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. Heat fixation kills some bacteria but is mostly used to affix the bacteria to the slide so that they don't rinse out during the staining procedure.
- The addition of iodide, which binds to crystal violet and traps it in the cell
- Rapid decolorization with ethanol or acetone

- *Counterstaining* with safranin. Carbol fuchsin is sometimes substituted for safranin since it more intensely stains anaerobic bacteria, but it is less commonly used as a counterstain.

GRAM-POSITIVE BACTERIA

Gram-positive bacteria generally have a single membrane (*monoderm*) surrounded by a thick peptidoglycan. This rule is followed by two phyla: *Firmicutes* (except for the classes Mollicutes and Negativicutes) and the *Actinobacteria*. In contrast, members of the Chloroflexi (green non-sulfur bacteria) are monoderms but possess a thin or absent (class Dehalococcoidetes) peptidoglycan and can stain negative, positive or indeterminate; members of the *Deinococcus–Thermus* group stain positive but are diderms with a thick peptidoglycan.

Historically, the Gram-positive forms made up the phylum *Firmicutes*, a name now used for the largest group. It includes many well-known genera such as *Lactobacillus*, *Bacillus*, *Listeria*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Clostridium*. It has also been expanded to include the *Mollicutes*; bacteria like *Mycoplasma*, *Thermoplasma* that lack cell walls and so cannot be Gram-stained, but are derived from such forms.

Some bacteria have cell walls which are particularly adept at retaining stains. These will appear positive by Gram stain even though they are not closely related to other Gram-positive bacteria. These are called acid-fast bacteria, and can only be differentiated from other Gram-positive bacteria by special staining procedures.

GRAM-NEGATIVE BACTERIA

Gram-negative bacteria generally possess a thin layer of peptidoglycan between two membranes (*diderms*). Most bacterial phyla are Gram-negative, including the cyanobacteria, green sulfur bacteria, and most *Proteobacteria* (exceptions being some members of the *Rickettsiales* and the insect-endosymbionts of the *Enterobacteriales*).

2.3 REFERENCES

- Colco, R (2005). "Gram Staining". *Current Protocols in Microbiology*. **00** (1): Appendix 3C. doi:10.1002/9780471729259.mca03cs00.
- Practical Medical Microbiology by Hams H. Hashem.

- U.S. National Library of medicine (Medline Plus**).**
- https://www.healthline.com/health/vdrl-test#procedure.
- Some important and latest topics taken from Google website.
- Hand book of Microbiology Society.
- Rana S.V.S, Biotechniques, Theory and Practices

Unit 3 Biotechniques (exercise based on chart/ picture or sample instrument)

CONTENT

3.1 Determination of pH using pH meter.

3.2 Demonstration of functioning of spectrophotometer.

3.3 Demonstration of use of bright field, phase contrast, dark field, fluorescence, confocal and electron microscopes (on photograph basis).

EXPERIMENT NO-01

OBJECTIVE: To demonstrate the pH using by pH meter.

INTRODUCTION

A **pH Meter** is a scientific instrument that measures the hydrogen-ion concentration (or pH) in a solution, indicating its acidity or alkalinity.

The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode. It usually has a glass electrode plus a calomel reference electrode, or a combination electrode.

In addition to measuring the pH of liquids, a special probe is sometimes used to measure the pH of a **pH Meter** is a scientific instrument that measures the hydrogen-ion concentration (or pH) in a solution, indicating its acidity or alkalinity.

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In addition to measuring the pH of liquids, a special probe is sometimes used to measure the pH of semi-solid substances.

Uses

- Knowledge of pH to greater or lesser accuracy is useful or critical in a great many situations, including of course chemical laboratory work.
- pH meters of various types and quality can be used for soil measurements in agriculture; water quality for water supply systems, swimming pools, etc.; brewing, industrially or domestically; healthcare, to ensure that solutions are safe when applied to patients or lethal as sterilants and disinfectants; and many other applications.

Circuit and operation

- Simple potentiometric pH meters simply measure the voltage between two electrodes and display the result converted into the corresponding pH value.
- They comprise a simple electronic amplifier and a pair of probes, or a combination probe, and some form of display calibrated in pH.
- The probe is the key part: it is a rod-like structure usually made of glass, with a bulb containing the sensor at the bottom.
- Frequent calibration with solutions of known pH, perhaps before each use, ensures the best accuracy. To measure the pH of a solution, the probe is dipped into it.

Probe care and cleaning

- Probes need to be kept clean of contamination as far as possible, and not touched by hand.
- Probes are best kept moist with a medium appropriate for the particular probe (distilled water, which can encourage diffusion out of the electrode, is undesirable) when not in use.

- If the bulb becomes contaminated with use it can be cleaned in the manner recommended by the manufacturer; a quick rinse in distilled water immediately after use, blotted (not wiped) off may be sufficient.
- One maker of laboratory-grade equipment gives different cleaning instructions for general cleaning (15' soak in a solution of bleach and detergent), salt (hydrochloric acid solution followed by sodium hydroxide and water), grease (detergent or methanol), clogged reference junction (KCl solution), protein deposits (pepsin and HCl, 1% solution), and air bubbles.

Calibration and use

- For very precise work the pH meter should be calibrated before each measurement. For normal use calibration should be performed at the beginning of each day.
- The reason for this is that the glass electrode does not give a reproducible e.m.f. over longer periods of time
- Calibration should be performed with at least two standard buffer solutions that span the range of pH values to be measured.
- For general purposes buffers at pH 4.00 and pH 10.00 are acceptable.
- The pH meter has one control (calibrate) to set the meter reading equal to the value of the first standard buffer and a second control which is used to adjust the meter reading to the value of the second buffer.
- A third control allows the temperature to be set.
- Standard buffer sachets, which can be obtained from a variety of suppliers, usually state how the buffer value changes with temperature.
- For more precise measurements, a three buffer solution calibration is preferred. As pH 7 is essentially, a "zero point" calibration (akin to zeroing or taring a scale or balance), calibrating at pH 7 first, calibrating at the pH closest to the point of interest (e.g. either 4 or 10) second and checking the third point will provide a more linear accuracy to what is essentially a non-linear problem.

- Some meters will allow a three-point calibration and that is the preferred scheme for the most accurate work. Higher quality meters will have a provision to account for temperature coefficient correction, and high-end pH probes have temperature probes built in.
- The calibration process correlates the voltage produced by the probe (approximately 0.06 volts per pH unit) with the pH scale.
- After each single measurement, the probe is rinsed with distilled water or deionized water to remove any traces of the solution being measured, blotted with a scientific wipe to absorb any remaining water which could dilute the sample and thus alter the reading, and then quickly immersed in a solution suitable for storage of the particular probe type.

TYPES OF PH METERS

A simple pH meter

pH meters range from simple and inexpensive pen-like devices to complex and expensive laboratory instruments with computer interfaces and several inputs for indicator and temperature measurements to be entered to adjust for the variation in pH caused by temperature. Specialty meters and probes are available for use in special applications, harsh environments, etc.

There are also holographic pH sensors, which allow pH measurement calorimetrically.

Figure 3.1 a simple pH meter

Figure 3.2 a Digital pH meter

HISTORY

The concept of pH was defined in 1909 by S. P. L. Sorensen, and electrodes were used for pH measurement in the 1920s.

In October 1934 Arnold Orville Beckman registered the first patent for a complete chemical instrument for the measurement of pH, U.S. Patent No. 2,058,761, for his "acidometer", later renamed the pH meter.

Beckman developed the prototype as an assistant professor of chemistry at the California Institute of Technology, when asked to devise a quick and accurate method for measuring the acidity of lemon juice for the California Fruit Growers Exchange (Sunkist). On April 8, 1935, Beckman's renamed National Technical Laboratories focused on the making of scientific instruments, with the Arthur H.

Thomas Company as a distributor for its pH meter.131–135. In its first full year of sales, 1936, the company sold 444 pH meters for $60,000 in sales. In years to come, it would bring in millions.

Radiometer in Denmark was founded in 1935, and began marketing a pH meter for medical use around 1936, but "the development of automatic pH-meters for industrial purposes was neglected.

Instead American instrument makers successfully developed industrial pH-meters with a wide variety of applications, such as in breweries, paper works, alum works, and water treatment systems. In 2004 the Beckman pH meter was designated an ACS National Historic Chemical Landmark in recognition of its significance as the first commercially successful electronic pH meter.

In the 1970s Jenco Electronics of Taiwan designed and manufactured the first portable digital pH meter. This meter was sold under Cole-Parmer's label.

Building a pH meter

A basic pH meter essentially measures the potential difference between two electrodes and displays the result calibrated in pH; the electronic circuit is very simple and easily built with a few cheap standard electronic components, plus the specialized pH probe.

1. SCOPE

- This test method is the procedure for determining the pH of water or soil samples by use of a pH meter. However, if the pH of any industrial by-product material (e.g.: cinders, flyash, etc.) is required, the procedure under 3.B. will be followed.

2. APPARATUS AND MATERIALS

- A 0.1 pt. (50 mL), wide-mouth glass beaker with a watch glass for cover. If lightweight material is to be tested, it may be necessary to increase beaker size up to a maximum of 0.5 pt. (250 mL).
- A pH meter, suitable for laboratory or field analysis, with either one or two electrodes.
- Standard buffer solutions of known pH values - standards to be used are pH of 4.0, 7.0, and 10.0.
- Distilled water
- A teaspoon or small scoop
- A thermometer capable of reading 77±18°F (25±10°C) to the nearest 0.1°C.
- A ¼ in. (6.3 mm) sieve conforming to the requirements of AASHTO Designation M-92-91 and a pan.
- A glass stirring rod
- A scale, minimum capacity of 1.1 lb. (500 g). It shall be accurate to 0.1% and be readable to 0.1 g.

*3. **PROCEDURE***

A. Water pH Determination

- Stir the water sample vigorously using a clean glass stirring rod.
- Pour a 40 mL ± 5 mL sample into the glass beaker using the watch glass for a cover.
- Let the sample stand for a minimum of one hour to allow the temperature to stabilize, stirring it occasionally while waiting. Measure the temperature of the sample and adjust the temperature controller of the pH meter to that of the sample temperature. This adjustment should be done just prior to testing.
- On meters with an automatic temperature control, follow the manufacturer's instructions.
- Standardize the pH meter by means of the standard solutions provided. Temperature and adjustments must be performed as stated.

- Immerse the electrode(s) of the pH meter into the water sample and turn the beaker slightly to obtain good contact between the water and the electrode(s).
- The electrode(s) require immersion 30 seconds or longer in the sample before reading to allow the meter to stabilize. If the meter has an auto read system, it will automatically signal when stabilized.
- Read and record the pH value to the nearest tenth of a whole number. If the pH meter reads to the hundredth place, a round off rule will apply as follows: If the hundredth place digit is less than 5, leave the tenth place digit as is. If it is greater than 5, round the tenth place digit up one unit. If the hundredth place digit equals 5, round the tenth place digit to the nearest even number.
- Rinse the electrode(s) well with distilled water, then dab lightly with tissues to remove any film formed on the electrode(s). Caution: Do not wipe the electrodes, as this may result in polarization of the electrode and consequent slow response.

B. SOIL pH DETERMINATION

The material must be separated on the ¼ in. (6.3 mm) sieve. Only the minus ¼ in. (6.3 mm) material is to be used for testing.

- Weigh and place 30±0.1 g of soil into the glass beaker.
- Add 30±0.1 g of distilled water to the soil sample. Stir to obtain soil slurry and then cover with watch glass.
- The sample must stand for a minimum of one hour, stirring every 10 to 15 minutes. This is to allow the pH of the soil slurry to stabilize.
- After one hour, the temperature of the sample should be stabilized. Measure the temperature of the sample and adjust the temperature controller of the pH meter to that of the sample temperature. This adjustment should be done just prior to testing. On meters with an automatic temperature control, follow the manufacturer's instructions.
- Standardize the pH meter by means of the standard solutions provided. Temperature and adjustments must be performed as stated.
- Immediately before immersing the electrode(s) into the sample, stir the sample well with a glass rod. Place the electrode(s) into the soil slurry solution and gently turn beaker to make good contact between the solution and the electrode(s). DO NOT place electrode(s) into the soil; only into the soil slurry solution.

- The electrode(s) require immersion 30 seconds or longer in the sample before reading to allow the meter to stabilize. If the meter has an auto read system, it will automatically signal when stabilized.
- Read and record the pH value to the nearest tenth of a whole number. If the pH meter reads to the hundredth place, a round off rule will apply as follows: If the hundredth place digit is less than 5, leave the tenth place digit as is. If it is greater than 5, round the tenth place digit up one unit. If the hundredth place digit equals 5, round the tenth place digit to the nearest even number.
- Rinse the electrode(s) well with distilled water, then dab lightly with tissues to remove any film formed on the electrode(s). Caution: Do not wipe the electrodes as this may result in polarization of the electrode(s) and consequent slow response.

NOTE 1 - To standardize the pH meter, use the 7.0 pH buffer standard solutions plus the other standard solution which is nearest the estimated pH value of the sample to be tested. If the manufacturer's instructions indicate a method other than that noted above, then those instructions must be followed.

NOTE 2 - When immersing electrode(s) into the glass beaker, care should be taken not to hit the bottom or side, causing damage to electrode(s).
NOTE 3 - If polarization does occur, as indicated by a slow response, rinse the electrode(s) and dab lightly again.

4. *PRECAUTIONS*

- Periodically check for damage to electrode(s).
- Electrode tips should be kept moist during storage. Follow manufacturer's instructions.

EXPERIMENT NO-02

***OBJECTIVE*:** To demonstrate the functioning of Spectrophotometer.

INTRODUCTION

In addition to the light microscope, the spectrophotometer is usually found in the biological laboratory. This instrument allows the investigator to identify compounds of biological interest and quantify them. Identification is determined by producing an absorption spectrum of a compound. Quantifying the amount of the material present, usually in solution, is done:

(a) directly if the substance is a strong absorber of a measurable wavelength ( ) of light; (b) indirectly by chemically modifying the compound so that it is a strong absorber at a measurable wavelength of light; or

(c) Indirectly by stoichiometrically coupling its reaction to the formation of another light absorbing compound.

MATERIALS

2,6-dichlorophenol indophenol, graduated cylinders, flasks, stir plates, stir bars, micropipettors, spectrophotometer tubes, rulers, spectrophotometer.

A BEER-LAMBERT LAW

Spectrophotometry, or spectrophotometric analysis, refers to the quantitative determination of the radiant energy ratio of incident to transmitted light beams at a given wavelength.

Spectrophotometers are instruments that allow for the determination of this ratio. Spectrophotometers are designed to make these measurements over a given range of wavelengths of the electromagnetic spectrum.

Usually, in the biological laboratory a spectrophotometer will allow for measurements in the UV and visible wavelengths.

If $\mathbf{P_i}$ is the intensity of the incident beam of light and $\mathbf{P_t}$ is the intensity of the transmitted light, then, by definition, the ratio P_t/P_i equals the **transmittance**, **T**, and log P_i/P_t equals the **absorbance, A**. Thus:

$$\mathbf{A = - \log T} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{(1)}$$

A fundamental law of spectrophotometry is the Beer-Lambert law, or simply Beer's Law that states that the amount of radiant energy absorbed (log P_i/P_t) by a compound in solution is proportional to its concentration and the length of the path that the light beam passes through the solution:

$$\mathbf{\log P_i / P_t = A = acl \ldots\ldots\ldots\ldots\ldots\ldots (2)}$$

Where:

c = concentration

l = path length

a = proportionality constant called **absorptivity**.

The absorptivity at a given wavelength is dependent upon the chemical structure of the compound, which determines the probability that the wavelength of light will be absorbed. The absorptivity constant is sometimes referred to as the **extinction coefficient.**

If the concentration is expressed in molarity, then **a** is called molar absorptivity or **molar extinction coefficient (e)**. Remember that **a** or **e** is dependent upon the wavelength. Conventionally, the path length used is 1 cm, and thus, the units for **e** are cm^{-1} M^{-1}. Note that **A**, therefore, is a unit less value, which it should be, based on its definition above (i.e., a ratio).

A plot of the absorbance of a substance in solution at a given wavelength, as a function of the molar amount of the substance present, is called a calibration plot or **standard curve.** If you examine equation #2, you will see that the slope of a standard curve is **e**. Having determined **e**, and knowing l (the path length is 1 cm), the concentration of the substance in any other similar sample can be determined using equation #2, Beer's Law.

An **absorbance spectrum** of a given compound is a graphical representation of the absorbance at each wavelength over a given wavelength range (usually UV - visible), that is, **A** vs. 8. Chemically different compounds have their own distinctive absorbance spectrum. You will be determining an absorbance spectrum for the electron acceptor 2, 6-

dichlorophenol inophenol (DCPIP) and will also plot absorbance spectra in a future lab exercise on plant pigments.

B. The Instrument

There are four essential parts to a spectrophotometer:

1. Light source
2. Monochromator or filter
3. Sample cell with holder
4. Detector

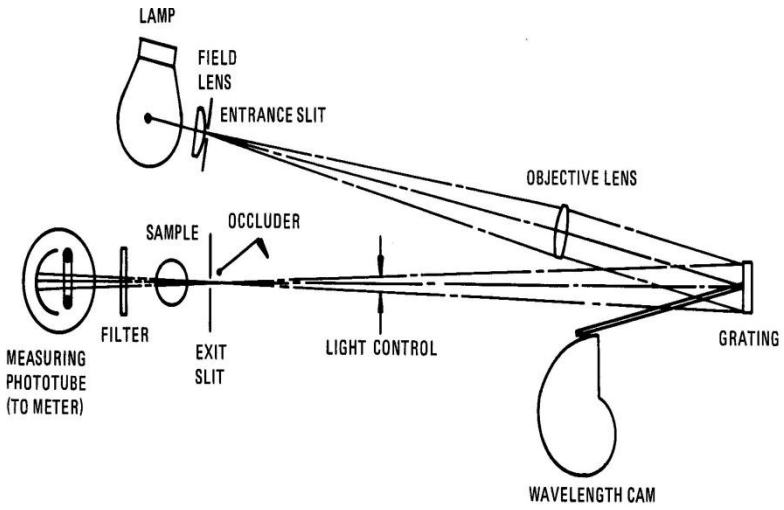


Figure 3.3.Essential parts of the spectrophotometer

The detector converts the radiant energy of the transmitted light into an electrical signal, the strength of which is proportional to the intensity of light transmitted (actually the number of photons striking its surface). A phototube is often used for this purpose. The electronics of the spectrophotometer convert this signal into a reading of transmittance or absorbance.

- Light sources do not emit the same light intensity over the entire spectrum; indeed, each source has its own inherent **emission spectrum.**
- Therefore, when determining an absorbance spectrum, it is important to adjust for the difference in light intensity at each wavelength of incident light.
- This can be done manually (using a "*ZERO CONTROL*" knob) or automatically, depending on the sophistication of the instrument.

C. Directions for Spectronic 20 Genesys ™ Spectrophotometer

1. Choose **A** from the **A/T/C** button to select for absorbance mode.
2. Press nm to select for correct wavelength.
3. Insert your blank into the cell holder and close the sample door.
4. Press the **0 ABS/100 %T** button to zero the absorbance.
5. Remove blank and insert your sample into the cell holder. Close door, and read absorbance from the LED display.

D. Preparing a standard curve and calculating the molar extinction coefficient

1. Prepare 100 ml of 1 mM 2, 6-dichlorophenol indophenol (DCPIP) in distilled water (DW) using the glassware provided. Make sure all the DCPIP goes into solution. *(The molecular weight of DCPIP is 290.08. g mole^{-1})*
2. Dilute the solution 1:10 by adding 10 ml of 1 mM DCPIP to 90 ml of DW to form a 100 M stock solution of DCPIP.
3. Prepare a serial dilution series of different concentrations of DCPIP in the spectrophotometer tubes provided. Take care to label each tube with a marker pen to indicate the appropriate concentration. Remember to use a new pipette tip for each manipulation.
4. Check that the dilution curve is correct by eye. Tube #9 should be the deepest blue in color.
5. Adjust setting to 500 nm. Zero the spectrophotometer using tube #1.

6. Remove tube 1 and place tube 9 in the spectrophotometer and record the absorbance value.

7. Repeat steps 5 and 6 at 10-nm increments from 500-700 nm. Zero at each wavelength before taking a measurement. Record values in Table. Plot these data (absorbance: y-axis; : x-axis) on the graph paper provided and determine the absorbance maximum, the wavelength at which DCPIP reaches its highest absorbance.

8. Set the wavelength to the absorbance maximum and readjust the zero control at this wavelength.

9. Determine the absorbance of tubes #1-9 at this wavelength. Record values in Table and construct a standard curve on the graph paper provided.

10. Determine the molar extinction coefficient for the dye from your standard curve. Enter this value below, for it will be used in a later laboratory exercise.

Micromolar Extinction Coefficient DCPIP @ _____ nm = _____

Wavelength (nm)	Absorbance
500	
510	
520	
530	
540	

550	
560	
570	
580	
590	
600	
610	
620	
630	
640	
650	
660	
670	
680	
690	
700	

Table: 1 Absorbance of Tube 9 as a Function of Wavelength

Tube	Absorbance (at $_{max}$)	ml of stock solution	ml distilled water	Final concentration of DCPIP (µM)
1		**0.0**	**5.0**	**0**
2		**0.05**	**4.95**	**1.0**
3		**0.25**	**4.75**	**5.0**
4		**0.5**	**4.50**	**10.0**
5		**1.0**	**4.0**	**20.0**

6		**1.5**	**3.5**	**30.0**
7		**2.5**	**2.5**	**50.0**
8		**4.0**	**1.0**	**80.0**
9		**5.0**	**0.0**	**100.0**

Table 2 Absorbance at $_{max}$ as a Function of DCPIP Concentration

11. You are provided with an additional sample containing an unknown concentration of DCPIP. Using your standard curve from part 9, determine the concentration of your unknown.

Unknown _____ Unknown absorbance _____

Unknown Concentration _____

12. knowing the molar extinction coefficient for DCPIP, Determine the concentration of your unknown Using Beer's law. Recall that A = acl
Calculated "unknown" concentration using Beer's law _____

- In chemistry, spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.
- It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.
- Spectrophotometry uses photometers that can measure a light beam's intensity as a function of its color (wavelength) known as spectrophotometers.
- Important features of spectrophotometers are spectral bandwidth (the range of colors it can transmit through the test sample), the percentage of sample-transmission, the logarithmic range of sample-absorption, and sometimes a percentage of reflectance measurement.
- A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases.

- However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200 nm - 2500 nm using different controls and calibrations.
- Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the **photometric determination.**

An example of an experiment in which spectrophotometry is used is the determination of the equilibrium constant of a solution. A certain chemical reaction within a solution may occur in a forward and reverse direction where reactants form products and products break down into reactants. At some point, this chemical reaction will reach a point of balance called an equilibrium point. In order to determine the respective concentrations of reactants and products at this point, the light transmittance of the solution can be tested using spectrophotometry. The amount of light that passes through the solution is indicative of the concentration of certain chemicals that do not allow light to pass through.

- The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology.
- They are widely used in many industries including semiconductors, laser and optical manufacturing, printing and forensic examination, and as well in laboratories for the study of chemical substances.
- Ultimately, a spectrophotometer is able to determine, depending on the control or calibration, what substances are present in a target and exactly how much through calculations of observed wavelengths.

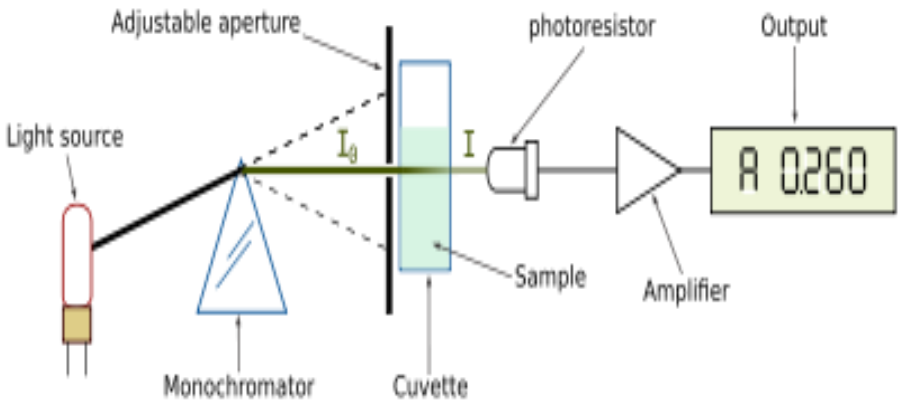


Figure 3.4 Single beam spectrophotometer

There are two major classes of devices: single beam and double beam.

- A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample.
- A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted.
- Although comparison measurements from double-beam instruments are easier and more stable, single-beam instruments can have a larger dynamic range and are optically simpler and more compact.
- Additionally, some specialized instruments, such as spectrophotometers built onto microscopes or telescopes, are single-beam instruments due to practicality.

Historically, spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum.

The grating can either be movable or fixed. If a single detector, such as a photomultiplier tube or photodiode is used, the grating can be scanned stepwise so that the detector can measure the light intensity at each wavelength (which will correspond to each "step"). Arrays of detectors, such as charge coupled devices (CCD) or photodiode arrays (PDA) can also be used.

In such systems, the grating is fixed and the intensity of each wavelength of light is measured by a different detector in the array. Additionally, most modern mid-infrared spectrophotometers use a Fourier transform technique to acquire the spectral information. The technique is called Fourier transform infrared spectroscopy.

When making transmission measurements, the spectrophotometer quantitatively compares the fraction of light that passes through a reference solution and a test solution, then electronically compares the intensities of the two signals and computes the percentage of transmission of the sample compared to the reference standard. For reflectance measurements, the spectrophotometer quantitatively compares the fraction of light that reflects from the reference and test samples.

Light from the source lamp is passed through a monochromator, which diffracts the light into a "rainbow" of wavelengths and outputs narrow bandwidths of this diffracted spectrum through a mechanical slit on the output side of the monochromator. These bandwidths are transmitted through the test sample.

Then the photon flux density (watts per metre squared usually) of the transmitted or reflected light is measured with a photodiode, charge coupled device or other light sensor. The transmittance or reflectance value for each wavelength of the test sample is then compared with the transmission or reflectance values from the reference sample. Most instruments will apply a logarithmic function to the linear transmittance ratio to calculate the 'absorbency' of the sample, a value which is proportional to the 'concentration' of the chemical being measured.

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is shone into a monochromator, diffracted into a rainbow, and split into two beams. It is then scanned through the sample and the reference solutions.
2. Fractions of the incident wavelengths are transmitted through, or reflected from, the sample and the reference.
3. The resultant light strikes the photodetector device, which compares the relative intensity of the two beams.
4. Electronic circuits convert the relative currents into linear transmission percentages and/or absorbance/concentration values.

Many older spectrophotometers must be calibrated by a procedure known as "zeroing", to balance the null current output of the two beams at the detector. The transmission of a reference substance is set as a baseline (datum) value, so the transmissions of all other substances are recorded relative to the initial "zeroed" substance. The spectrophotometer then converts the transmission ratio into 'absorbency', the concentration of specific components of the test sample relative to the initial substance.

APPLICATIONS IN BIOCHEMISTRY

Spectrophotometry is an important technique used in many biochemical experiments that involve DNA, RNA, and protein isolation, enzyme kinetics and biochemical analyses.

A brief explanation of the procedure of spectrophotometry includes comparing the absorbency of a blank sample that does not contain a colored compound to a sample that contains a colored compound.

The spectrophotometer is used to measure colored compounds in the visible region of light (between 350 nm and 800 nm), thus it can be used to find more information about the substance being studied.

In biochemical experiments, a chemical and/or physical property is chosen and the procedure that is used is specific to that property in order to derive more information about the sample, such as the quantity, purity, enzyme activity, etc.

Spectrophotometry is also a helpful procedure for protein purification and can also be used as a method to create optical assays of a compound. Because a spectrophotometer measures the wavelength of a compound through its color, a dye binding substance can be added so that it can undergo a color change and be measured.

Spectrophotometers have been developed and improved over decades and have been widely used among chemists. It is considered to be a highly accurate instrument that is also very sensitive and therefore extremely precise, especially in determining color change.

This method is also convenient for use in laboratory experiments because it is an inexpensive and relatively simple process.

UV-visible spectrophotometry

The most common spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near-infrared region as well.

Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science. It is a known fact that it operates best at the range of 0.2-0.8 O.D.

Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They take readings in the region of every 5–20 nanometers along the visible region, and produce a spectral reflectance curve or a data stream for alternative presentations.

These curves can be used to test a new batch of colorant to check if it makes a match to specifications, e.g., ISO printing standards.

Traditional visible region spectrophotometers cannot detect if a colorant or the base material has fluorescence. This can make it difficult to manage color issues if for example one or more of the printing inks is fluorescent.

Where a colorant contains fluorescence, a bi-spectral fluorescent spectrophotometer is used. There are two major setups for visual spectrum spectrophotometers, d/8 (spherical) and 0/45.

The names are due to the geometry of the light source, observer and interior of the measurement chamber. Scientists use this instrument to measure the amount of compounds in a sample.

If the compound is more concentrated more light will be absorbed by the sample; within small ranges, the Beer-Lambert law holds and the absorbance between samples vary with concentration linearly.

In the case of printing measurements two alternative settings are commonly used-without/with UV filter to control better the effect of UV brighteners within the paper stock.

Samples are usually prepared in cuvettes; depending on the region of interest, they may be constructed of glass, plastic (visible spectrum region of interest), or quartz (Far UV spectrum region of interest).

APPLICATIONS

- Estimating dissolved organic carbon concentration
- Specific Ultraviolet Absorption for metric of aromaticity
- Bial's Test for concentration of pentoses

Infrared Spectrophotometry

Spectrophotometers designed for the infrared region are quite different because of the technical requirements of measurement in that region.

One major factor is the type of photo sensors that are available for different spectral regions, but infrared measurement is also challenging because virtually everything emits IR light as thermal radiation, especially at wavelengths beyond about 5 μm.

Another complication is that quite a few materials such as glass and plastic absorb infrared light, making it incompatible as an optical medium. Ideal optical materials are salts, which do not absorb strongly.

Samples for IR spectrophotometry may be smeared between two discs of potassium bromide or ground with potassium bromide and pressed into a pellet. Where aqueous solutions are to be measured, insoluble silver chloride is used to construct the cell.

Spectroradiometers

Spectroradiometers, which operate almost like the visible region spectrophotometers, are designed to measure the spectral density of illuminants.

Applications may include evaluation and categorization of lighting for sales by the manufacturer, or for the customers to confirm the lamp they decided to purchase is within their specifications.

COMPONENTS

1. The light source shines onto or through the sample.
2. The sample transmits or reflects light.
3. The detector detects how much light was reflected from or transmitted through the sample.
4. The detector then converts how much light the sample transmitted or reflected into a number.

EXPERIMENT NO - 03

OBJECTIVE: To demonstrate of use of bright-field microscopy

INTRODUCTION

Bright-field microscopy is the simplest of all the optical microscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample.

Bright-field microscopy is the simplest of a range of techniques used for illumination of samples in light microscopes and its simplicity makes it a popular technique. The typical appearance of a bright-field microscopy image is a dark sample on a bright background, hence the name.

Light path

The light path of a bright-field microscope is extremely simple; no additional components are required beyond the normal light microscope setup. The light path therefore consists of:

- A transillumination light source, commonly a halogen lamp in the microscope stand;
- A condenser lens which focuses light from the light source onto the sample; and
- Objective lens which collects light from the sample and magnifies the image.
- Oculars and/or a camera to view the sample image

Bright field microscopy may use critical or Köhler illumination to illuminate the sample.

Performance

Bright-field microscopy typically has low contrast with most biological samples as few absorb light to a great extent. Staining is often required to increase contrast, which prevents use on live cells in many situations. Bright field illumination is useful for samples which have

an intrinsic colour, for example chloroplasts in plant cells.Comparison of transillumination techniques used to generate contrast in a sample of tissue paper. 1.559 μm/pixel. Bright field illumination, sample contrast comes from absorbance of light in the sample.

Cross-polarized light illumination, sample contrast comes from the rotation of polarized light through the sample. Dark field illumination, sample contrast comes from light scattered by the sample.

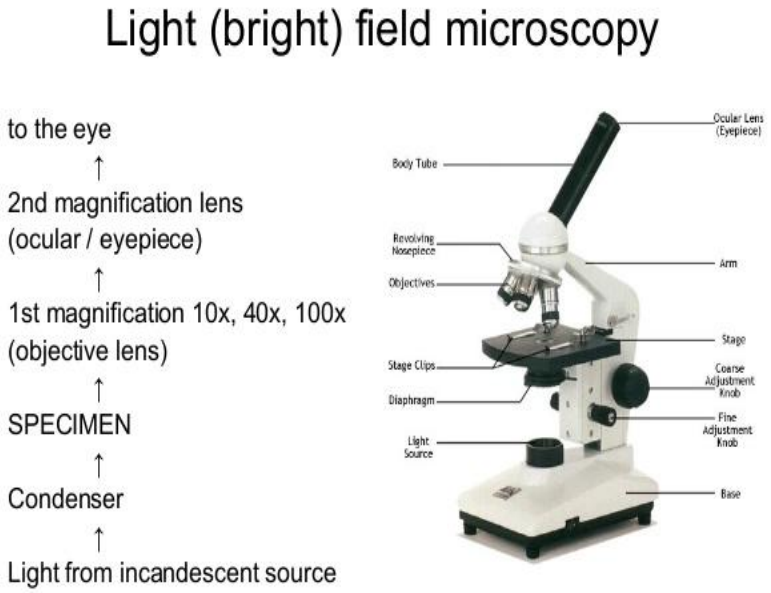


Figure 3.5 Light field microscopy

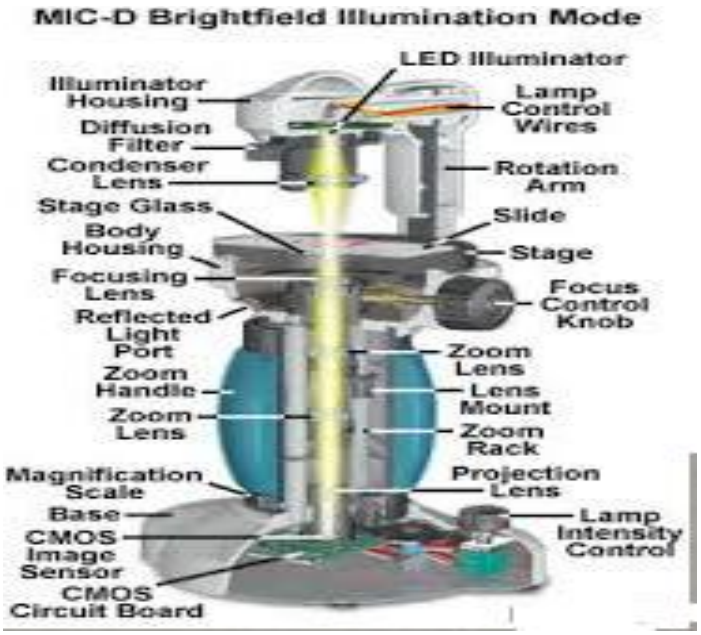


Figure 3.6 Bright field Illumination Mode

Phase contrast illumination, sample contrast comes from interference of different path lengths of light through the sample. Bright-field microscopy is a standard light microscopy technique, and therefore magnification is limited by the resolving power possible with the wavelength of visible light.

ADVANTAGES

- Simplicity of setup with only basic equipment required.

Limitations

- Very low contrast of most biological samples.
- Low apparent optical resolution due to the blur of out of focus material.
- Samples that are naturally colorless and transparent cannot be seen well, e.g. many types of mammalian cells.
- These samples often have to be stained before viewing. Samples that do have their own colour can be seen without preparation, e.g. the observation of cytoplasmic streaming in Chara cells.

Enhancements

- Reducing or increasing the amount of the light source via the iris diaphragm.
- Use of an oil immersion objective lens and special immersion oil placed on a glass cover over the specimen. Immersion oil has the same refraction as glass and improves the resolution of the observed specimen.
- Use of sample staining methods for use in microbiology, such as simple stains (Methylene blue, Safranin, Crystal violet) and differential stains (Negative stains, flagellar stains, endospore stains).
- Use of a colored (usually blue) or polarizing filter on the light source to highlight features not visible under white light. The use of filters is especially useful with mineral samples

EXPERIMENT NO – 3.1

OBJECTIVE: To demonstrate of use of Phase contrast microscopy

INTRODUCTION

Phase contrast microscopy, first described in 1934 by Dutch physicist Frits Zernike, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles).

In effect, the phase contrast technique employs an optical mechanism to translate minute variations in phase into corresponding changes in amplitude, which can be visualized as differences in image contrast.

One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without previously being killed, fixed, and stained.

As a result, the dynamics of ongoing biological processes can be observed and recorded in high contrast with sharp clarity of minute specimen detail.

In **f**igure **1** is a cut-away diagram of a modern upright phase contrast microscope, including a schematic illustration of the phase contrast optical train.

Partially coherent illumination produced by the tungsten-halogen lamp is directed through a collector lens and focused on a specialized annulus (labeled **condenser annulus**) positioned in the sub stage condenser front focal plane.

Wave fronts passing through the annulus illuminate the specimen and either passes through undeviated or is diffracted and retarded in phase by structures and phase gradients present in the specimen.

Undeviated and diffracted light collected by the objective is segregated at the rear focal plane by a **phase plate** and focused at the intermediate image plane to form the final phase contrast image observed in the eyepieces.

Prior to the invention of phase contrast techniques, transmitted bright field illumination was one of the most commonly utilized observation modes in optical microscopy, especially for fixed, stained specimens or other types of samples having high natural absorption of visible light.

Collectively, specimens readily imaged with bright field illumination are termed **amplitude objects** (or specimens) because the amplitude or intensity of the illuminating wave fronts is reduced when light passes through the specimen.

The addition of phase contrast optical accessories to a standard bright field microscope can be employed as a technique to render a contrast-enhancing effect in transparent specimens that is reminiscent of optical staining.

Light waves that are diffracted and shifted in phase by the specimen (termed a **phase object**) can be transformed by phase contrast into amplitude differences that are observable in the eyepieces.

Large, extended specimens are also easily visualized with phase contrast optics due to diffraction and scattering phenomena that occur at the edges of these objects.

The performance of modern phase contrast microscopes is so refined that it enables specimens containing very small internal structures, or even just a few protein molecules, to be detected when the technology is coupled to electronic enhancement and post-acquisition image processing.

In figure is a comparison of living cells in culture imaged in both bright field and phase contrast illumination.

The cells are human glial brain tissue grown in monolayer culture bathed with a nutrient medium containing amino acids, vitamins, mineral salts, and fetal calf serum.

In bright field illumination, the cells appear semi-transparent with only highly refractive regions, such as the membrane, nucleus, and unattached cells (rounded or spherical), being visible.

When observed using phase contrast optical accessories, the same field of view reveals significantly more structural detail. Cellular attachments become discernable, as does much of the internal structure. In addition, the contrast range is dramatically improved.

Interaction of Light Waves with Phase Specimens

An incident wave front present in an illuminating beam of light becomes divided into two components upon passing through a phase specimen.

The primary component is an undeviated (or undiffracted; **zeroth-order**) planar wave front, commonly referred to as the **surround** (**S**) wave, which passes through and around the specimen, but does not interact with it.

In addition, a deviated or **diffracted** spherical wave front (**D**-wave) is also produced, which becomes scattered over a wide arc (in many directions) that passes through the full aperture of the objective.

After leaving the specimen plane, surround and diffracted light waves enter the objective front lens element and are subsequently focused at the intermediate image plane where they combine through interference to produce a resultant **particle** wave (often referred to as a **P**-wave). The mathematical relationship between the various light waves generated in phase contrast microscopy can be described simply as:

Formula 1 - Relationship between Various Light Waves Generated in Phase Contrast Microscopy P = S + D

Detection of the specimen image depends on the relative intensity differences, and therefore on the amplitudes, of the particle and surround (**P** and **S**) waves.

If the amplitudes of the particle and surround waves are significantly different in the intermediate image plane, then the specimen acquires a considerable amount of contrast and is easily visualized in the microscope eyepieces.

Otherwise, the specimen remains transparent and appears as it would under ordinary bright field conditions (in the absence of phase contrast or other contrast-enhancing techniques).

In terms of optical path variations between the specimen and its surrounding medium, the portion of the incident light wave front that traverses the specimen (**D**-wave), but does not pass through the surrounding medium (**S**-wave), is slightly retarded.

For arguments in phase contrast microscopy, the role of the specimen in altering the optical path length (in effect, the relative phase shift) of waves passing through is of paramount importance.

In classical optics, the optical path length (**OPL**) through an object or space is the product of the refractive index (**n**) and the thickness (**t**) of the object or intervening medium as described by the relationship:

Formula 2 - Optical Path Length

Optical Path Length (OPL) = $n \times t$

When light passes from one medium into another, the velocity is altered proportionally to the refractive index differences between the two media.

Thus, when a coherent light wave emitted by the focused microscope filament passes through a phase specimen having a specific thickness (**t**) and refractive index (**n**), the wave is either increased or decreased in velocity.

If the refractive index of the specimen is greater than that of the surrounding medium, the wave is reduced in velocity while passing through the specimen and is subsequently retarded in relative phase when it emerges from the specimen.

In contrast, when the refractive index of the surrounding medium exceeds that of the specimen, the wave is advanced in phase upon exiting the specimen. The difference in location of an emergent wave front between the specimen and surrounding medium is termed the **phase shift** (δ) and is defined in radians as:

Formula 3 - Phase Shift $\delta = 2\pi\Delta/\lambda$

In the equation above, the term **D** is referred to as the **optical path difference**, which is similar to the optical path length:

Formula 4 - Optical Path Difference

Optical Path Difference (OPD) = $\Delta = (n_2 - n_1) \times t$

Where **n(2)** is the refractive index of the specimen and **n(1)** is the refractive index of the surrounding medium.

The optical path difference results from the product of two terms: the thickness of the specimen, and its difference in refractive index with the surrounding medium.

In many cases, the optical path difference can be quite large even though the thickness of the specimen is small.

On the other hand, when the refractive index of the specimen equals that of the surrounding medium, the optical path difference is zero regardless of whether the specimen thickness is large or small.

Wave Interactions in Phase Contrast Microscopy

Phase relationships between the surround, diffracted, and particle (**S**, **D**, and **P**) waves in the region of the specimen at the image plane for bright field microscopy (in the absence of phase contrast optical accessories) are presented in figure.

The surround and particle waves, whose relative amplitudes determine the amount of specimen contrast, are illustrated as red and green lines (respectively).

The wave produced by diffraction from the specimen, which is never directly observed, is depicted as a blue wave of lower amplitude.

The surround and diffracted waves recombine through interference to generate the resultant particle wave in the image plane of the microscope.

The amplitude of each wave illustrated in figure represents the sum of the electric vectors of the individual component waves.

Interpretation of Phase Contrast Images

Images produced by phase contrast microscopy are relatively simple to interpret when the specimen is thin and distributed evenly on the substrate (as is the case with living cells grown in monolayer tissue culture).

When thin specimens are examined using positive phase contrast optics, which is the traditional form produced by most manufacturers, they appear darker than the surrounding medium when the refractive index of the specimen exceeds that of the medium.

Phase contrast optics differentially enhance the contrast near the edges surrounding extended specimens, such as the boundary between a cellular membrane and the bathing nutrient medium, and produce overall high-contrast images that can be roughly interpreted as density maps.

Because the amplitude and intensity of a specimen image in phase contrast is related to refractive index and optical path length, image density can be utilized as a gauge for approximating relationships between various structures.

In effect, a series of internal cellular organelles having increasing density, such as vacuoles, cytoplasm, the inter phase nucleus, and the nucleolus (or mitotic chromosomes), are typically visualized as progressively darker objects relative to a fixed reference, such as the background.

It should also be noted that numerous optical artifacts are present in all phase contrast images, and large extended specimens often present significant fluctuations in contrast and image intensity.

Symmetry can also be an important factor in determining how both large and small specimens appear in the phase contrast microscope.

Sensible interpretation of phase contrast images requires careful scrutiny and examination to ensure that artifacts are not incorrectly assigned to important structural features.

For example, some internal cellular organelles and components often have a lower refractive index than that of the surrounding cytoplasm, while others have a higher refractive index.

Because of the varying refractive indices exhibited by these numerous intracellular structures, the interior of living cells, when viewed in a positive phase contrast microscope, can reveal an array of intensities ranging from very bright to extremely dark.

For example, pinocytotic vesicles, lipid droplets, and air vacuoles present in plants and single cell protozoan's have a lower refractive index than the cytoplasm, and thus appear brighter than other components. In contrast, as discussed above, organelles that have high refractive indices (nuclei, ribosome, mitochondria, and the nucleolus) appear dark in the microscope. If the phase retardation introduced by the specimen is large enough (a phase shift of the diffracted wave by approximately a half-wavelength), interference between the diffracted waves and the surround waves becomes constructive, rendering these specimens brighter than the surrounding background.

In order to avoid confusion regarding bright and dark contrast in phase contrast images, the optical path differences occurring within the specimen preparation should be carefully considered.

As discussed above, the optical path difference is derived from the product of the refractive index and the specimen (object) thickness, and is related to the relative phase shift between the specimen and background (diffracted and surround) waves.

It is impossible to distinguish between high and low refractive index components in a phase contrast image without information pertaining to the relative thickness of the components.

For example, a small specimen having a high refractive index can display an identical optical path difference to a larger specimen having a lower refractive index. The two specimens will have approximately the same intensity when viewed through a phase contrast optical system.

In many biological experiments, conditions that produce a shrinking or swelling of cells or organelles can result in significant contrast variations. The external medium can also be replaced with another having either a higher or lower refractive index to generate changes in specimen image contrast.

In fact, the effect on image contrast of refractive index variations in the surrounding medium forms the basis.

Fig 3.8 *Dark field and phase contrast microcopies operating principle*

Fig.3.9 A phase-contrast microscope

WORKING PRINCIPLE

The basic principle to making phase changes visible in phase-contrast microscopy is to separate the illuminating (background) light from the specimen-scattered light (which makes up the foreground details) and to manipulate these differently.

The ring-shaped illuminating light (green) that passes the condenser annulus is focused on the specimen by the condenser. Some of the illuminating light is scattered by the specimen (yellow).

The remaining light is unaffected by the specimen and forms the background light (red). When observing an unstained biological specimen, the scattered light is weak and typically phase-shifted by −90° (due to both the typical thickness of specimens and the refractive index difference between biological tissue and the surrounding medium) relative to the background light.

This leads to the foreground (blue vector) and background (red vector) having nearly the same intensity, resulting in low image contrast.

In a phase-contrast microscope, image contrast is increased in two ways: by generating constructive interference between scattered and background light rays in regions of the field of view that contain the specimen, and by reducing the amount of background light that reaches the image plane.

First, the background light is phase-shifted by −90° by passing it through a phase-shift ring, which eliminates the phase difference between the background and the scattered light rays.

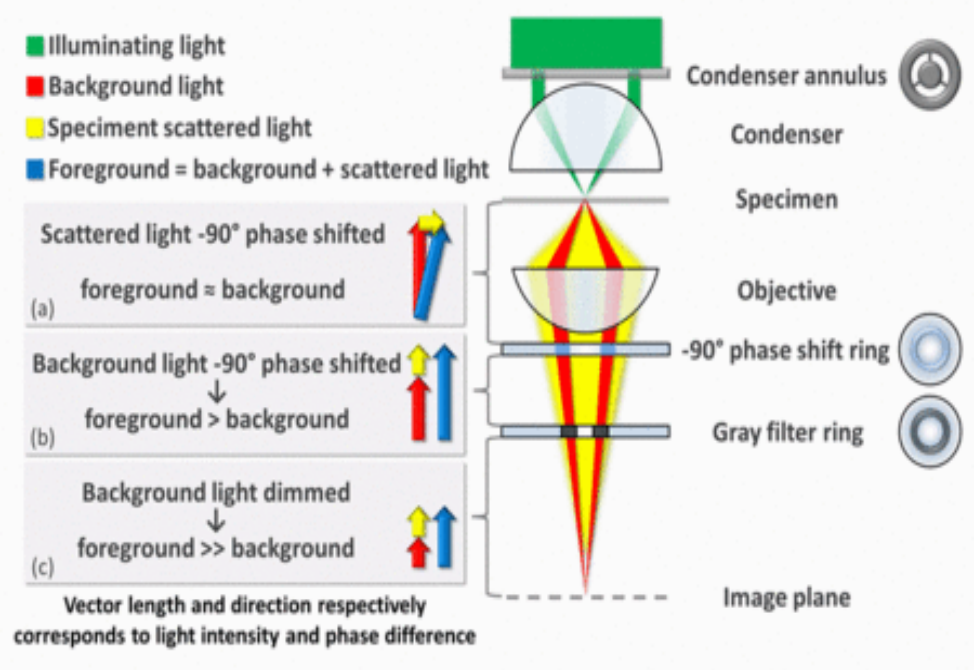


Fig 3.10 Operating principle dark field and phase contrast microcopies

EXPERIMENT NO- 3.2

OBJECTIVE: To demonstrate of use of dark field microscopy

INTRODUCTION

Have you ever heard of a dark field microscope? While such a name may sound like a sci-fi gadget used to measure black holes, in reality it's just a handy tool used to view certain types of translucent samples.

The average microscope user may not know about the concept of dark field microscopy, yet it can shed new light on the old way of viewing specimens.

Most people who have survived a biology class know what a light field microscope is. This type of scope uses bright field illumination, meaning it floods the specimen with white light from the condenser without any interference.

Thus the specimen shows up as a dark image on a light background (or white field if you will).

This type of unit works best with specimens that have natural color pigments. The samples need to be thick enough to absorb the incoming light; so staining is usually paired with this type of microscope.

Yet what if the specimen is light colored or translucent, like the plankton on the right? It certainly won't stand out against a strong white background. Additionally, some specimens are just too thin.

They cannot absorb any of the light that passes through them, so they appear invisible to the user. This is where the concept of dark field illumination comes in!

Rather than using direct light from the condenser, one uses an opaque disk to block the light into just a few scattered beams.

Now the background is dark, and the sample reflects the light of the beams only. This results in a light colored specimen against a dark background (dark field), perfect for viewing clear or translucent details.

On a grand scale, the same thing happens every day when you look up at the sky. Do the stars disappear when it's light out? Of course not! They're still there, their brilliance blotted out by the mid-day sun.

If you're still having a hard time visualizing this concept, think of a dusty room with the light on and the door open. You may feel the dust affecting your breathing, but you probably won't see it flying through the air.

Now turn off the light and close the door to just a sliver, while leaving the light on in the adjacent room. If you look at that sliver of light coming through the door, you'll see all sorts of dust motes suspended in it. You're employing a similar principle when you use dark field illumination!

Use

Dark field microscopes are used in a number of different ways to view a variety of specimens that are hard to see in a light field unit.

Live bacteria, for example, are best viewed with this type of microscope, as these organisms are very transparent when unstained.

There are multitudes of other ways to use dark field illumination, often when the specimen is clear or translucent. Some examples:

- Living or lightly stained transparent specimens
- Single-celled organisms
- Live blood samples
- Aquatic environment samples (from seawater to pond water)
- Living bacteria
- Hay or soil samples
- Pollen samples
- Certain molecules such as caffeine crystals (right)

Dark field microscopy makes many invisible specimens appear visible. Most of the time the specimens invisible to bright field illumination are living, so you can see how important it is to bring them into view!

ADVANTAGES AND DISADVANTAGES

No one system is perfect and dark field microscopy may or may not appeal to you depending on your needs. Some advantages of using a dark field microscope are:

- Extremely simple to use
- Inexpensive to set up (instructions on how to make your own dark field microscope are below)
- Very effective in showing the details of live and unstained samples

Some of the disadvantages are:

- Limited colors (certain colors will appear, but they're less accurate and most images will be just black and white)
- Images can be difficult to interpret to those unfamiliar with dark field microscopy
- Although surface details can be very apparent, the internal details of a specimen often don't stand out as much with a dark field setup.

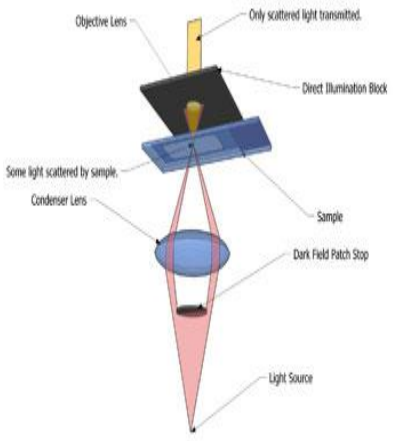


Fig 3.11Contrasting examples of dark field illumination

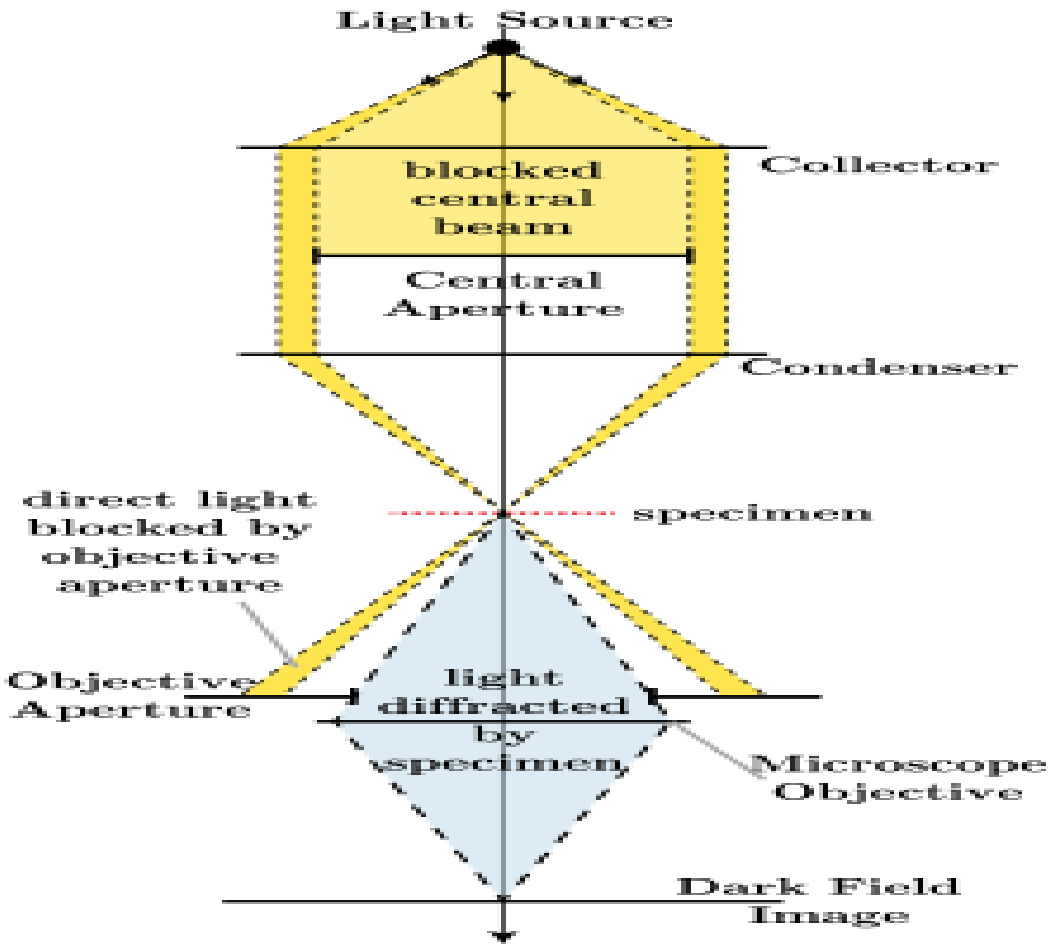


Fig 3.12 Diagram illustrating the light path through a dark field microscope

Digital dark field analysis

This a mathematical technique intermediate between direct and reciprocal (Fourier-transform) space for exploring images with well-defined periodicities, like electron microscope lattice-fringe images.

As with analog dark field imaging in a transmission electron microscope,it allows one to "light up" those objects in the field of view where periodicities of interest reside.

Unlike analog dark field imaging it may also allow one to map the Fourier-phase of periodicities, and hence phase-gradients which provide quantitative information on vector lattice-strain.

Experiment No- 3.3

OBJECTIVE: To demonstrate of use of Fluorescent microscopy

INTRODUCTION

The absorption and subsequent re-radiation of light by organic and inorganic specimens is typically the result of well-established physical phenomena described as being either **fluorescence** or **phosphorescence**.

The emission of light through the fluorescence process is nearly simultaneous with the absorption of the excitation light due to a relatively short time delay between photon absorption and emission, ranging usually less than a microsecond in duration.

When emission persists longer after the excitation light has been extinguished, the phenomenon is referred to as phosphorescence.

A fluorescence microscope is much the same as a conventional light microscope with added features to enhance its capabilities.

- The conventional microscope uses visible light (400-700 nanometers) to illuminate and produce a magnified image of a sample.
- A fluorescence microscope, on the other hand, uses a much higher intensity light source which excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source.

Fluorescent microscopy is often used to image specific features of small specimens such as microbes. It is also used to visually enhance 3-D features at small scales.

This can be accomplished by attaching fluorescent tags to anti-bodies that in turn attach to targeted features, or by staining in a less specific manner.

When the reflected light and background fluorescence is filtered in this type of microscopy the targeted parts of a given sample can be imaged.

This gives an investigator the ability to visualize desired organelles or unique surface features of a sample of interest. Confocal fluorescent microscopy is most often used to accentuate the 3-D nature of samples.

This is achieved by using powerful light sources, such as lasers, that can be focused to a pinpoint. This focusing is done repeatedly throughout one level of a specimen after another.

Most often an image reconstruction program pieces the multi level image data together into a 3-D reconstruction of the targeted sample.

Fig 3.13Fluorescent Microscopy

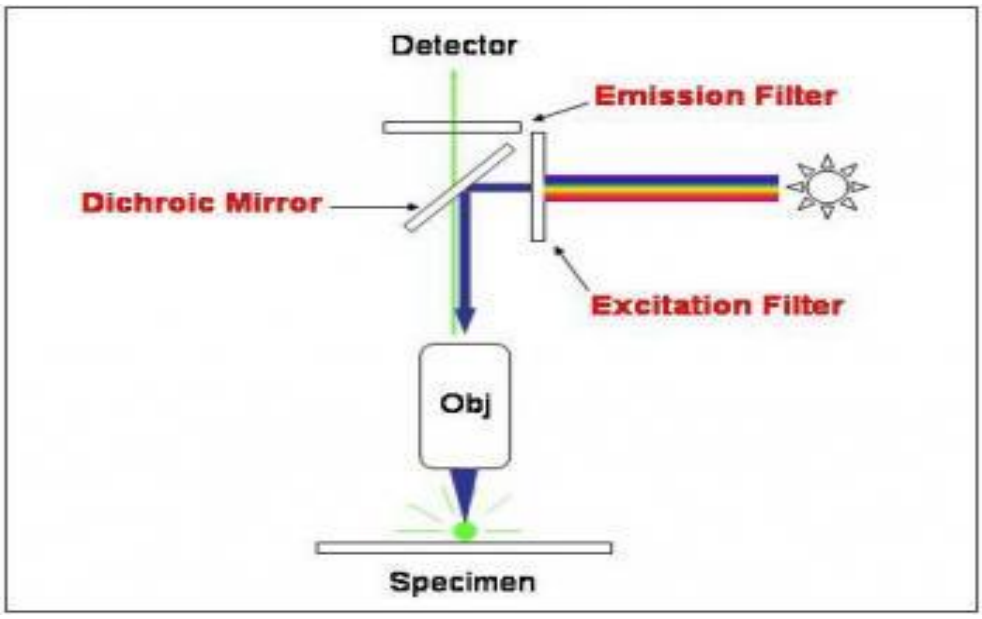


Figure 3.14 showing the filters and mirror in a fluorescent microscope

How does Fluorescent Microscopy Work

In most cases the sample of interest is labeled with a fluorescent substance known as a fluorophore and then illuminated through the lens with the higher energy source.

The illumination light is absorbed by the fluorophores (now attached to the sample) and causes them to emit a longer lower energy wavelength light.

This fluorescent light can be separated from the surrounding radiation with filters designed for that specific wavelength allowing the viewer to see only that which is fluorescing.

The basic task of the fluorescence microscope is to let excitation light radiate the specimen and then sort out the much weaker emitted light from the image.

First, the microscope has a filter that only lets through radiation with the specific wavelength that matches your fluorescing material.

The radiation collides with the atoms in your specimen and electrons are excited to a higher energy level.

When they relax to a lower level, they emit light. To become detectable (visible to the human eye) the fluorescence emitted from the sample is separated from the much brighter excitation light in a second filter.

This works because the emitted light is of lower energy and has a longer wavelength than the light that is used for illumination.

Most of the fluorescence microscopes used in biology today is epi-fluorescence microscopes, meaning that both the excitation and the observation of the fluorescence occur above the sample.

Most use a Xenon or Mercury arc-discharge lamp for the more intense light source.

APPLICATIONS

The refinement of epi-fluorescent microscopes and advent of more powerful focused light sources, such as lasers, has led to more technically advanced scopes such as the confocal laser scanning microscopes and total internal reflection fluorescence microscopes (TIRF).

CLSM's are invaluable tools for producing high resolution 3-D images of subsurfaces in specimens such as microbes.

Their advantage is that they are able to produce sharp images of thick samples at various depths by taking images point by point and reconstructing them with a computer rather than viewing whole images through an eyepiece.

These microscopes are often used for -

- Imaging structural components of small specimens, such as cells
- Conducting viability studies on cell populations (are they alive or dead?)
- Imaging the genetic material within a cell (DNA and RNA)
- Viewing specific cells within a larger population with techniques such as FISH

Fluorescence Light Sources

An unfortunate consequence of low emission levels in most fluorescence microscopy applications is that the number of photons that reach the eye or camera detector is also very low.

In most cases, the collection efficiency of optical microscopes is less than 30 percent and the concentration of many fluorophores in the optical path ranges in the micromolar or nanomolar regions.

In order to generate sufficient excitation light intensity to produce detectable emission, powerful compact light sources, such as high-energy short arc-discharge lamps, are necessary.

The most common lamps are mercury burners, ranging in wattage from 50 to 200 Watts, and the xenon burners that range from 75 to 150 Watts.

These light sources are usually powered by an external direct current supply, furnishing enough start-up power to ignite the burner through ionization of the gaseous vapor and to keep it burning with a minimum of flicker.

The microscope arc-discharge lamp external power supply is usually equipped with a timer to track the number of hours the burner has been in operation.

Arc lamps lose efficiency and are more likely to shatter if used beyond their rated lifetime (200-300 hours).

The mercury burners do not provide even intensity across the spectrum from ultraviolet to infrared, and much of the intensity of the lamp is expended in the near ultraviolet.

Prominent peaks of intensity occur at 313, 334, 365, 406, 435, 546, and 578 nanometers. At other wavelengths in the visible light region, the intensity is steady although not nearly so bright (but still useable in most applications).

In considering illumination efficiency, mere lamp wattage is not the prime consideration. Instead, the critical parameter is the mean luminance must be considered, taking into account the source brightness, arc geometry, and the angular spread of emission.

Table 1 - Luminous Density of Selected Light Sources

Lamp	**Current (Amperes)**	**Luminous Flux (Lumens)**	**Mean Luminous Density (cd/mm2)**	**Arc Size (H x W) (Millimeters)**
Mercury Arc (100 Watt)	5	2200	1700	0.25 x 0.25

Xenon Arc (75 Watt)	5.4	850	400	0.25 x 0.50
Xenon Arc (500 Watt)	30	9000	3500	0.30 x 0.30
Tungsten Halogen	8	2800	45	4.2 x 2.3

CONCLUSIONS

The modern fluorescence microscope combines the power of high performance optical components with computerized control of the instrument and digital image acquisition to achieve a level of sophistication that far exceeds that of simple observation by the human eye.

Microscopy now depends heavily on electronic imaging to rapidly acquire information at low light levels or at visually undetectable wavelengths.

These technical improvements are not mere window dressing, but are essential components of the light microscope as a system.

The era when optical microscopy was purely a descriptive instrument or an intellectual toy is past.

At present, optical image formation is only the first step toward data analysis. The microscope accomplishes this first step in conjunction with electronic detectors, image processors, and display devices that can be viewed as extensions of the imaging system.

Computerized control of focus, stage position, optical components, shutters, filters, and detectors is in widespread use and enables experimental manipulations that were not humanly possible with mechanical microscopes.

The increasing application of electro-optics in fluorescence microscopy has led to the development of optical tweezers capable of manipulating sub-cellular structures or particles, the imaging of single molecules, and a wide range of sophisticated spectroscopic applications.

EXPERIMENT NO- 3.4

OBJECTIVE: To demonstrate of use of confocal microscopy

INTRODUCTION

This microscopy facility provides users with the opportunity to prepare and image their own samples.

The EM/Confocal Specialist will provide technical support to new users, faculty, graduate students, and staff, for specimen preparation and will provide comprehensive training to operate the microscopes.

Confocal microscopy, most frequently **confocal laser scanning microscopy** (**CLSM**), is an optical imaging technique for increasing optical resolution and contrast of a micrograph by means of adding a spatial pinhole placed at the confocal plane of the lens to eliminate out-of-focus light.

It enables the reconstruction of three-dimensional structures from the obtained images by collecting sets of images at different depths (a process known as optical sectioning) within a thick object.

This technique has gained popularity in the scientific and industrial communities and typical applications are in life sciences, semiconductor inspection and materials science.

A conventional microscope "sees" as far into the specimen as the light can penetrate, while a confocal microscope only "sees" images one depth level at a time. In effect, the CLSM achieves a controlled and highly limited depth of focus.

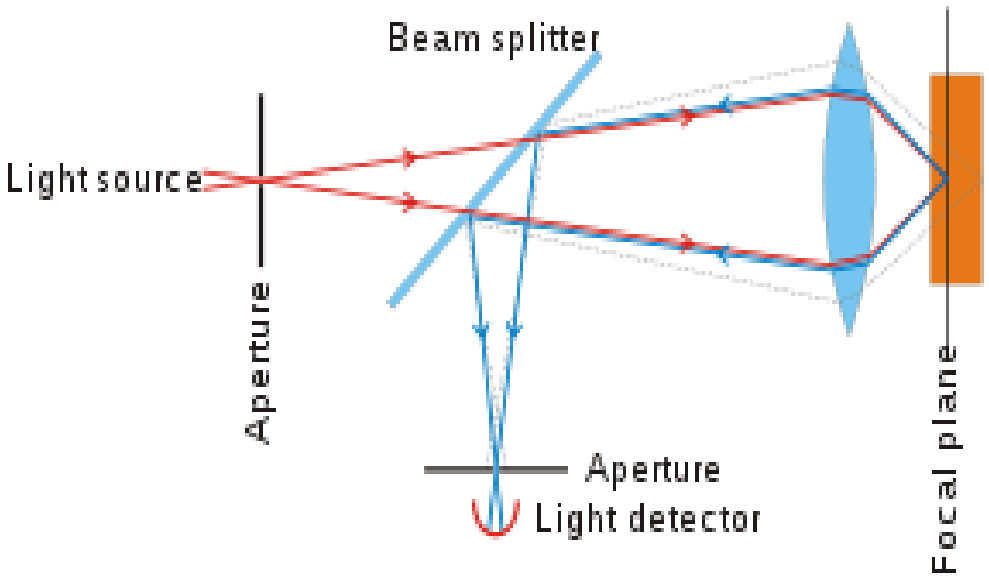


Fig 3.15Principle of confocal microscopy

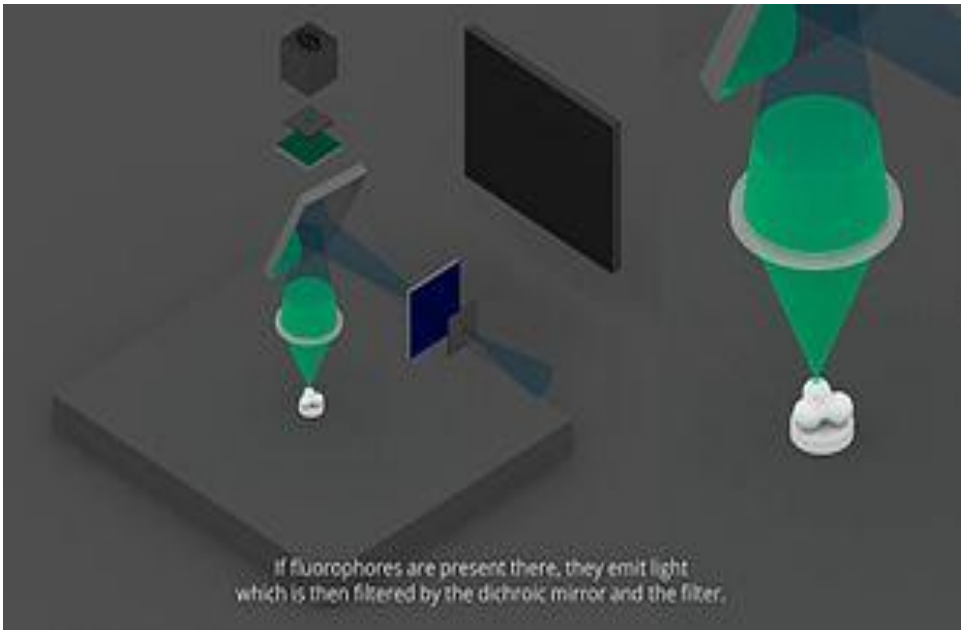
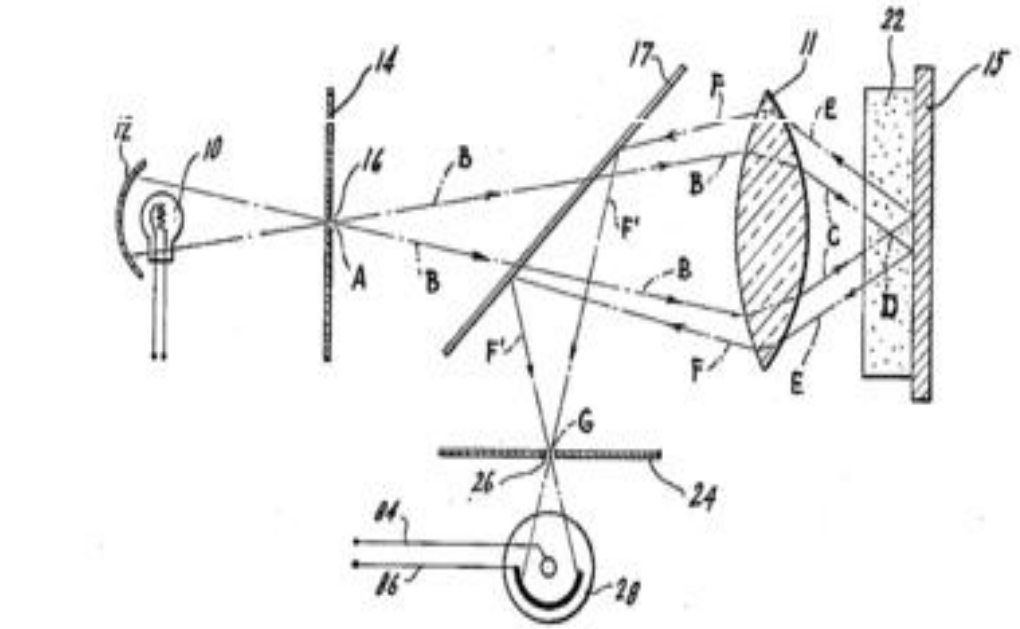


Fig 3.16 Fluorescent and confocal microscopes operating principle



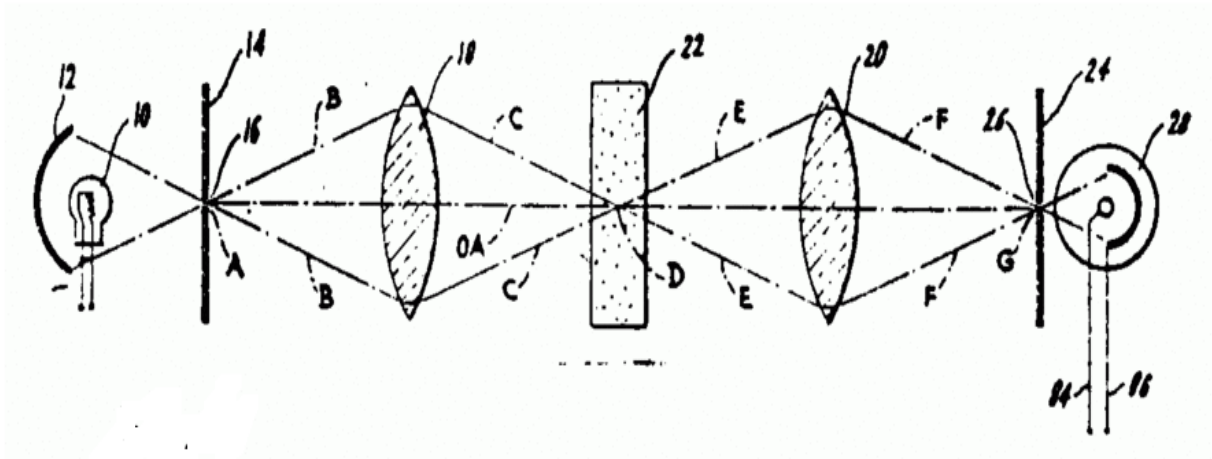


Fig 3.17Confocal point sensor principle

EXPERIMENT NO- 3.5

OBJECTIVE: To demonstrate of use of Electron microscopy

INTRODUCTION

- An **electron microscope** is a microscope that uses a beam of accelerated electrons as a source of illumination.
- As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects.
- A transmission electron microscope can achieve better than 50 pm resolution and magnifications of up to about 10,000,000 xs whereas most light microscopes are limited by diffraction to about 200 nm resolution and useful magnifications below 2000x.
- Transmission electron microscopes use electrostatic and electromagnetic lenses to control the electron beam and focus it to form an image.
- These electron optical lenses are analogous to the glass lenses of an optical light microscope.
- Electron microscopes are used to investigate the ultra structure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals.
- Industrially, electron microscopes are often used for quality control and failure analysis.
- Modern electron microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the image.

The first electromagnetic lens was developed in 1926 by Hans Busch. According to Dennis Gabor, the physicist Leó Szilárd tried in 1928 to convince Busch to build an electron microscope, for which he had filed a patent.

German physicist Ernst Ruska and the electrical engineer Max Knoll constructed the prototype electron microscope in 1931, capable of four-hundred-power magnification; the apparatus was the first demonstration of the principles of electron microscopy.

Two years later, in 1933, Ruska built an electron microscope that exceeded the resolution attainable with an optical (light) microscope.[Moreover, Reinhold Rudenberg, the scientific director of Siemens-Schuckertwerke, obtained the patent for the electron microscope in May 1931.

In 1932, Ernst Lubcke of Siemens & Halske built and obtained images from a prototype electron microscope, applying concepts described in the Rudenberg patent applications. Five years later (1937), the firm financed the work of Ernst Ruska and Bodo von Borries, and employed Helmut Ruska (Ernst's brother) to develop applications for the microscope, especially with biological specimens. Also in 1937, Manfred von Ardenne pioneered the scanning electron microscope. The first commercial electron microscope was produced in 1938 by Siemens.

Fig 3.18A modern transmission electron microscope

Types

Transmission electron microscope (TEM)

The original form of electron microscope, the transmission electron microscope (TEM) uses a high voltage electron beam to illuminate the specimen and create an image.

The electron beam is produced by an electron gun, commonly fitted with a tungsten filament cathode as the electron source.

The electron beam is accelerated by an anode typically at +100 keV (40 to 400 keV) with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam.

When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope.

The spatial variation in this information (the "image") may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide.

Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fibre optic light-guide to the sensor of a digital camera.

The image detected by the digital camera may be displayed on a monitor or computer.

The resolution of TEMs is limited primarily by spherical aberration, but new generations of aberration correctors have been able to partially overcome spherical aberration to increase resolution.

Hardware correction of spherical aberration for the high-resolution transmission electron microscopy (HRTEM) has allowed the production of images with resolution below 0.5 angstrom (50 picometres) and magnifications above 50 million times.

The ability to determine the positions of atoms within materials has made the HRTEM an important tool for nano-technologies research and development.

Transmission electron microscopes are often used in electron diffraction mode.

The advantages of electron diffraction over X-ray crystallography are that the specimen need not be a single crystal or even a polycrystalline powder, and also that the Fourier transform reconstruction of the object's magnified structure occurs physically and thus avoids the need for solving the phase problem faced by the X-ray crystallographers after obtaining their X-ray diffraction patterns of a single crystal or polycrystalline powder.

The major disadvantage of the transmission electron microscope is the need for extremely thin sections of the specimens, typically about 100 nanometers.

Biological specimens are typically required to be chemically fixed, dehydrated and embedded in a polymer resin to stabilize them sufficiently to allow ultrathin sectioning.

Sections of biological specimens, organic polymers and similar materials may require special treatment with heavy atom labels in order to achieve the required image contrast.

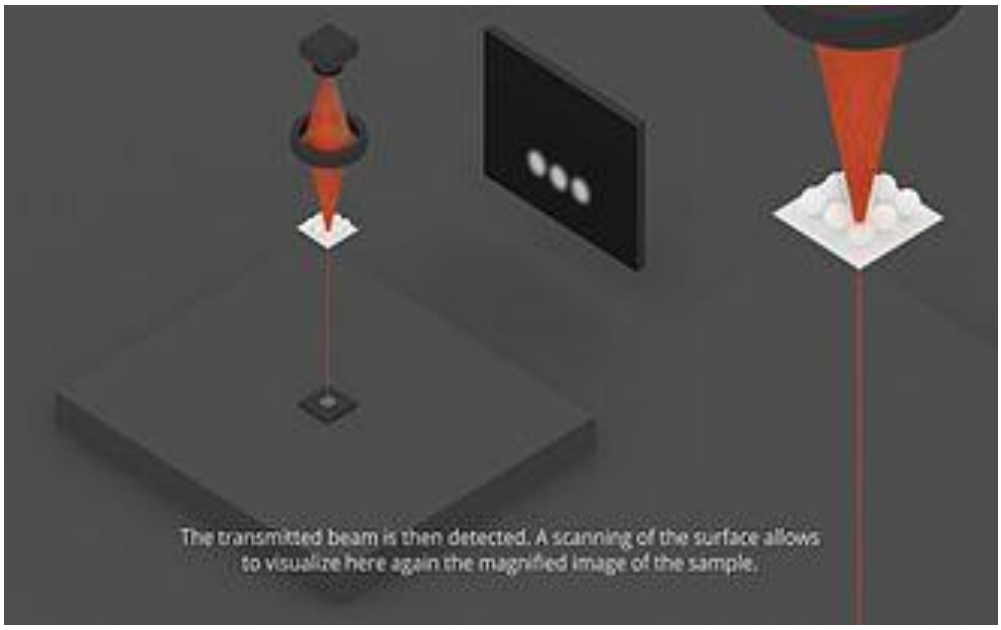


Fig 3.20 Operating principle of a Transmission Electron Microscope

Scanning electron microscope (SEM)

The SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen (raster scanning).

When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms.

The lost energy is converted into alternative forms such as heat, emission of low-energy secondary electrons and high-energy backscattered electrons, light emission (cathodoluminescence) or X-ray emission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition.

The image displayed by an SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated.

In the SEM image of an ant shown below and to the right, the image was constructed from signals produced by a secondary electron detector, the normal or conventional imaging mode in most SEMs.

Generally, the image resolution of an SEM is at least an order of magnitude poorer than that of a TEM.

However, because the SEM image relies on surface processes rather than transmission, it is able to image bulk samples up to many centimeters in size and (depending on instrument design and settings) has a great depth of field, and so can produce images that are good representations of the three-dimensional shape of the sample.

Another advantage of SEM is its variety called environmental scanning electron microscope (ESEM) can produce images of sufficient quality and resolution with the samples being wet or contained in low vacuum or gas.

This greatly facilitates imaging biological samples that are unstable in the high vacuum of conventional electron microscopes.

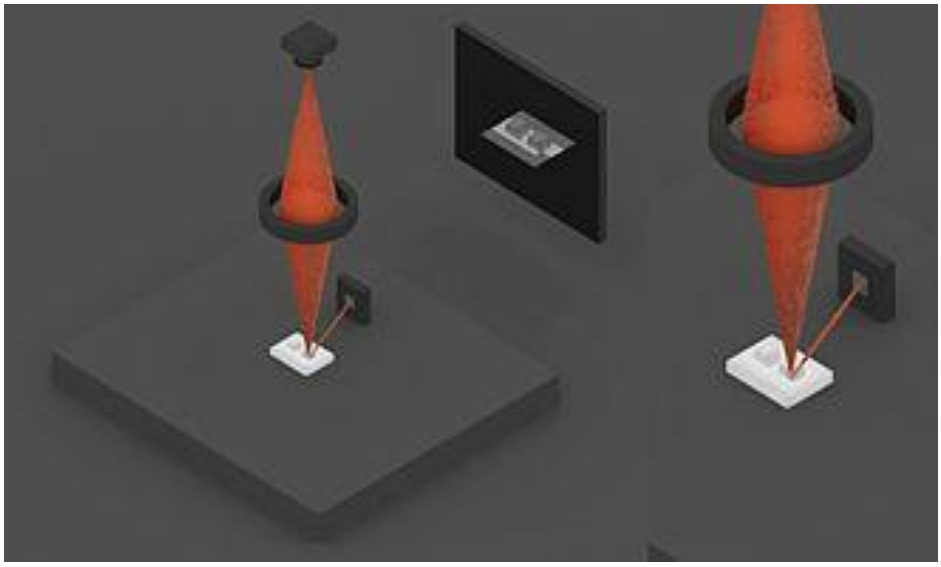


Fig 3.21Operating principle of a Scanning Electron Microscope

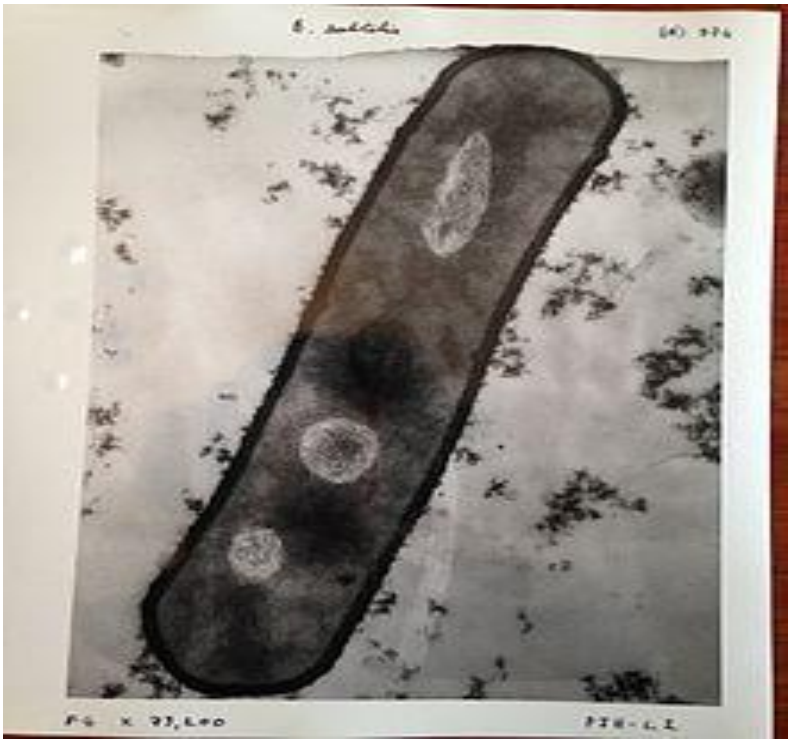


Fig 3.22Image of bacillus subtilis taken with a 1960s electron microscope

SUMMERY

1. A scientific instrument (pH meter, Spectrophotometer, Bright Field, Phase Contrast, Dark Field, Fluroscence, Cofocal and Electron Microscope) are an instrument used for laboratory purposes.

2. Most are measuring instruments. They may be specifically designed, constructed and refined for the purpose. Over time, instruments have become more accurate and precise.

3. Scientific instruments are part of laboratory equipment, but are considered more sophisticated and more specialized than other measuring instruments as scales, rulers, chronometers, thermometers or even waveform generators.

4. They are increasingly based upon the integration of computers to improve and simplify control, enhance and extend instrumental functions, conditions, parameter adjustments and data sampling, collection, resolution, analysis (both during and post-process), storage and retrieval.

GLOSSARY

1**. pH Meter**: Instrument that measures the hydrogen-ion concentration (or pH) in a solution, indicating its **acidity** or **alkalinity**. The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode.

2. **Cuvet(te):** A clear, rectangular vessel of glass or plastic used to hold solutions for spectrometry. Don't place its ribbed sides in the light path of the spectrometer!

3**. Absorbance and 100% Line**: Absorbance is the unit utilized for measuring the amount of IR radiation absorbed by a material.

4. **Wavelength and Wavenumber**. Wavelength is the interval between two adjacent crests or troughs of a light wave. Wave number is 1/wavelength and is expressed in cm^{-1}. It is widely utilized as the X axis unit in infrared spectra.

5. **Zero Path Difference (ZPD), or Zero Optical Path Difference (ZOPD):** In an interferometer, the mirror displacement upon which the optical path difference for the two beams is zero. The detector signal is often much larger at ZPD, ZOPD, and is called the center burst.

6. **TEM:** Transmission Electron Microscopy.

7. **SEM:** Scanning Electron Microscope.

8. **Dark-field microscope**. A **microscope** in which an object is illuminated only from the sides so that it appears bright against a **dark** background.

9. **Absorbance (Optical Density) -** The quantity of light absorbed by a chemical or biological substance as measured in a spectrophotometer or similar device. Units of absorbance are equivalent to the logarithm of the reciprocal transmittance (the ratio of the transmitted light intensity to incident light intensity).

10. **Bioluminescence -** A biochemical oxidative process that results in the release of energy as emitted light. Firefly luminescence, which requires the enzyme luciferase to catalyze a reaction between the substrate luciferin and molecular oxygen (in the presence of adenosine

triphosphate), is a commonly employed example of bioluminescence. The phenomenon occurs in a wide variety of marine organisms and insects.

SELF ASSESSMENT QUESTIONS

1. How often should I calibrate my pH sensor?
2. How can I calibrate the spectrophotometer?
3. What are the TEM and SEM?
4. What is optical density?
5. What are zero path differences?

REFERENCES

1. Advanced Light Microscopy vol. 1 Principles and Basic Properties by Maksymilian Pluta, Elsevier (1988)
2. Introduction to Light Microscopy by S. Bradbury, B. Bracegirdle, BIOS Scientific Publishers (1998)
3. Microbiology: Principles and Explorations by Jacquelyn G. Black, John Wiley & Sons, Inc. (2005).
4. Douglas B. Murphy - Department of Cell Biology and Anatomy and Microscope Facility, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, 107 WBSB, Baltimore, Maryland 21205.
5. Jump up ^ "The phase contrast microscope". Nobel Media AB.
6. Jump up ^ "Phase Contrast". Leica Science Lab.
7. Jump up ^ Frits Zernike (1942). "Phase contrast, a new method for the microscopic observation of transparent objects part I". Physica. 9 (7): 686–698. Bibcode:1942Phy.....9..686Z. Doi:10.1016/S0031-8914(42)80035-X.
8. Confocal Microscopy (3rd Ed.). Berlin: Springer. ISBN 0-387-25921-X.
9. Jump up ^ Vincze L (2005). "Confocal X-ray Fluorescence Imaging and XRF Tomography for Three Dimensional Trace Element Microanalysis". Microscopy and Microanalysis. 11 (Supplement 2). doi:10.1017/S1431927605503167.

SUGGESTED READINGS

1. Sharma, V.K.; Techniques in Microscopy and Cell Biology -Tata McGraw Hill.
2. Instrumentation and Control Systems- William Bolton
3. Instrumentation for Process Measurement and Control Norman A. Anderson.
4. Instrumentation for Engineers and Scientists (Textbooks in Electrical and Electronic Engineering) – by John D. Turner (Author), Martyn Hill (Author)

TERMINAL QUESTIONS

1. What are the working principles of pH meter?

2. Write biological applications of spectrophotometer.

3. Write short notes on:

   (i) SEM

   (ii) Absorbance

5. Write the significance of Confocal Microscope.
6. What is the use of dark field microscopy?

Unit 4 Biotechnology/Biotechniques

Content

4.1 Study of the principles and applications of the following equipment

4.1.1 Laminar flow

4.1.2 Autoclave

4.1.3 Elisa reader

4.1.4 PCR machine

4.1.5 Refrigrated centrifuge

4.1.6 Transilluminator

4.2 Double helical DNA modal

4.3 Chromatography or Thin Layer Chromatography (TLC)

4.4 Recombinant DNA techniques

Experiment No-(01) 4.1

***OBJECTIVE*:** Study of the principal and application of Laminar flow

INTRODUCTION

In fluid dynamics, **laminar flow** (or streamline flow) occurs when a fluid flows in parallel layers, with no disruption between the layers.

At low velocities, the fluid tends to flow without lateral mixing, and adjacent layers slide past one another like playing cards.

There are no cross-currents perpendicular to the direction of flow, nor eddies or swirls of fluids.

In laminar flow, the motion of the particles of the fluid is very orderly with particles close to a solid surface moving in straight lines parallel to that surface.

Laminar flow is a flow regime characterized by high momentum diffusion and low momentum convection.

When a fluid is flowing through a closed channel such as a pipe or between two flat plates, either of two types of flow may occur depending on the velocity and viscosity of the fluid: laminar flow or turbulent flow.

Laminar flow tends to occur at lower velocities, below a threshold at which it becomes turbulent.

Turbulent flow is a less orderly flow regime that is characterised by eddies or small packets of fluid particles which result in lateral mixing. In non-scientific terms, laminar flow is smooth while turbulent flow is rough.

Laminar flow barriers

Fig 4.1Experimental chamber for studying chemo taxis in response to laminar flow

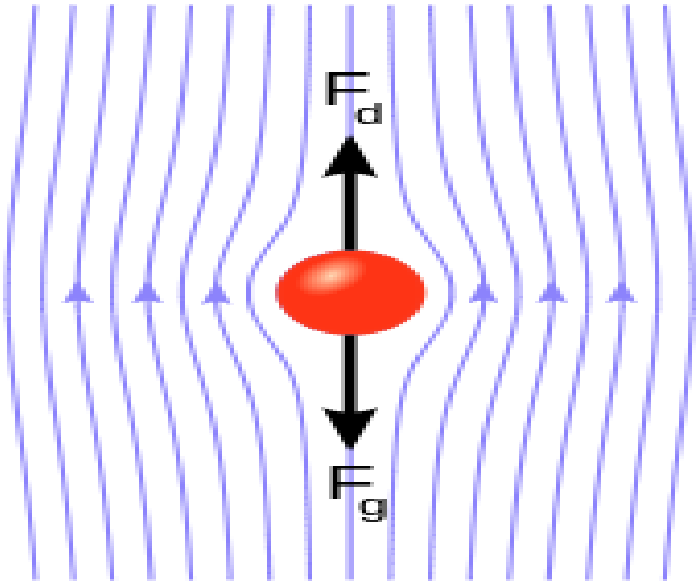


Fig 4.2 Reynolds Number

Relationship with the Reynolds number

The common example is flow through a pipe, where the Reynolds number is defined as:

$$\mathbf{Re} = \frac{\rho \mathbf{v} D_\mathrm{H}}{\mu} = \frac{\mathbf{v} D_\mathrm{H}}{\nu} = \frac{\mathbf{Q} D_\mathrm{H}}{\nu A}$$

Where:

- D_H is the hydraulic diameter of the pipe; its characteristic travelled length, L, (m).
- **Q** is the volumetric flow rate (m^3/s).
- A is the pipe's cross-sectional area (m^2).
- **v** is the mean velocity of the fluid (SI units: m/s).
- μ is the dynamic viscosity of the fluid (Pa·s = N·s/m^2 = kg/(m·s)).
- ν is the kinematic viscosity of the fluid, $\nu = \mu/\rho$ (m^2/s).
- ρ is the density of the fluid (kg/m^3).

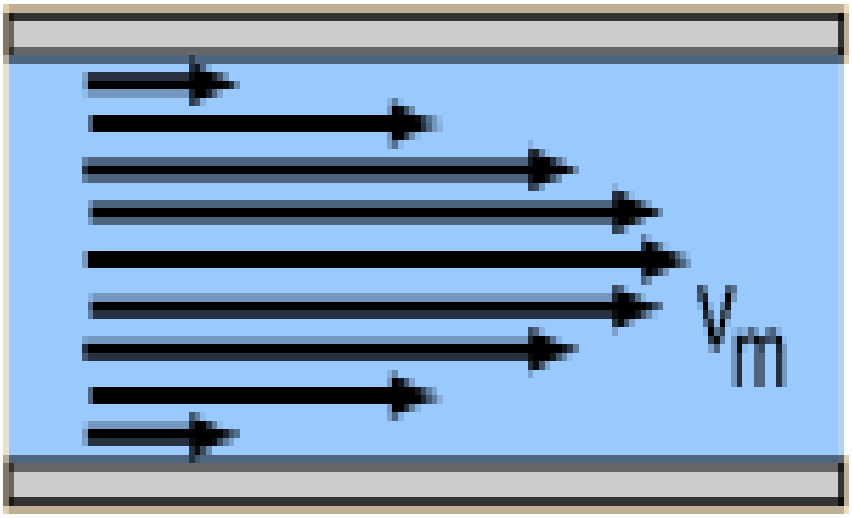
For such systems, laminar flow occurs when the Reynolds number is below a critical value of approximately 2,040; through the transition range is typically between 1,800 and 2,100.

For fluid systems occurring on external surfaces, such as flow past objects suspended in the fluid, other definitions for Reynolds numbers can be used to predict the type of flow around the object. The particle Reynolds number Re_p would be used for particle suspended in flowing fluids, for example.

As with flow in pipes, laminar flow typically occurs with lower Reynolds numbers, while turbulent flow and related phenomena, such as vortex shedding, occur with higher Reynolds numbers.

Examples:

In the case of a moving plate in a liquid, it is found that there is a layer (lamina) that moves with the plate, and a layer next to any stationary plate that is stationary.



EXPERIMENT NO- 4.2

***OBJECTIVE*:** Study of the principal and application of Autoclave

INTRODUCTION

An **autoclave** is a pressure chamber used to carry out industrial processes requiring elevated temperature and pressure different from ambient air pressure.

Autoclaves are used in medical applications to perform sterilization and in the chemical industry to cure coatings and vulcanize rubber and for hydrothermal synthesis.

They are also used in industrial applications, especially regarding composites, see autoclave (industrial).

Many autoclaves are used to sterilize equipment and supplies by subjecting them to high-pressure saturated steam at 121 °C (249 °F) for around 15–20 minutes depending on the size of the load and the contents.

The autoclave was invented by Charles Chamberland in 1879, although a precursor known as the steam digester was created by Denis Papin in 1679.

The name comes from Greek auto-, ultimately meaning self and Latin clavis meaning key, thus a self-locking device.

WHAT IS AN AUTOCLAVE?

The **autoclave** carries out that exact function of sterilizing materials.

It is a machine that uses pressure and steam to reach and maintain a temperature that is too high for any microorganisms or their spores to live.

Microorganisms are what most people commonly refer to as germs. These are the bacteria, viruses, fungi, parasites, etc. that are able to cause infections in our bodies.

Spores are the environment-resistant form of the microorganisms. Even though they are able to withstand harsher conditions, they still can be killed if extreme conditions are maintained for an extended period of time.

HOW IT WORKS

Autoclaves are pressure cookers very similar to the ones that you see in the stores. If you have used, or are familiar with pressure cookers, then you know that foods cook a lot faster in a pressure cooker than they do in a regular pot or in the oven. This is due to the intense heat and pressure that is applied to the food.

The same mechanism works against living microorganisms. Once an autoclave is started, steam is pushed into the chamber that contains the items that are being sterilized.

As the steam goes in, the pressure and temperature within the chamber is increased. Most autoclaves are set to increase steam pressure until a temperature of at least 121 degrees Celsius is reached (about 250 degrees Fahrenheit).

This temperature and pressure will remain at this level for at least 15 minutes. This is a high enough temperature for a long enough period of time to kill any and all microorganisms and their spores.

Uses

Sterilization autoclaves are widely used in microbiology, medicine, podiatry, tattooing, body piercing, veterinary medicine, mycology, funeral homes, dentistry, and prosthetics fabrication. They vary in size and function depending on the media to be sterilized.

Typical loads include laboratory glassware, other equipment and waste, surgical instruments, and medical waste.

Air removal

It is very important to ensure that all of the trapped air is removed from the autoclave before activation, as trapped air is a very poor medium for achieving sterility.

Steam at 134 °C can achieve in three minutes the same sterility that hot air at 160 °C can take two hours to achieve. Methods of air removal include:

Downward displacement (or **gravity-type**):

As steam enters the chamber, it fills the upper areas first as it is less dense than air. This process compresses the air to the bottom, forcing it out through a drain which often contains a temperature sensor.

Only when air evacuation is complete does the discharge stop. Flow is usually controlled by a steam trap or a solenoid valve, but bleed holes are sometimes used, often in conjunction with a solenoid valve.

As the steam and air mix, it is also possible to force out the mixture from locations in the chamber other than the bottom.

Steam pulsing

Pressurized and then depressurized to near atmospheric Air dilution by using a series of steam pulses, in which the chamber is alternately pressure.

Vacuum pumps

A vacuum pump sucks air or air/steam mixtures from the chamber.

Super atmospheric cycles

Achieved with a vacuum pump. It starts with a vacuum followed by a steam pulse followed by a vacuum followed by a steam pulse.

The number of pulses depends on the particular autoclave and cycle chosen.

Sub-atmospheric cycles

Similar to the super-atmospheric cycles, but chamber pressure never exceeds atmospheric pressure until they pressurize up to the sterilizing temperature.

A medical autoclave is a device that uses steam to sterilize equipment and other objects. This means that all bacteria, viruses, fungi, and spores are inactivated.

However, prions, such as those associated with Creutzfeldt–Jakob disease, may not be destroyed by autoclaving at the typical 134 °C for three minutes or 121 °C for 15 minutes.

Although that a wide range species of archaea, including *Geogemma barosii*, can survive at temperatures above 121 °C, no archaea are known to be infectious or pose a health risk to humans; in fact their biochemistry is so vastly different from our own and their multiplication rate is far too slow for microbiologists to worry about them.

Autoclaves are found in many medical settings, laboratories, and other places that need to ensure the sterility of an object.

Many procedures today employ single-use items rather than sterilizable, reusable items.

This first happened with hypodermic needles, but today many surgical instruments (such as forceps, needle holders, and scalpel handles) are commonly single-use rather than reusable items.

Autoclaves are of particular importance in poorer countries due to the much greater amount of equipment that is re-used.

Providing stove-top or solar autoclaves to rural medical centres has been the subject of several proposed medical aid missions.

Because damp heat is used, heat-labile products (such as some plastics) cannot be sterilized this way or they will melt.

Paper and other products that may be damaged by steam must also be sterilized another way.

In all autoclaves, items should always be separated to allow the steam to penetrate the load evenly.

Autoclaving is often used to sterilize medical waste prior to disposal in the standard municipal solid waste stream.

This application has become more common as an alternative to incineration due to environmental and health concerns raised because of the combustion by-products emitted by incinerators, especially from the small units which were commonly operated at individual hospitals.

Incineration or a similar thermal oxidation process is still generally mandated for pathological waste and other very toxic and/or infectious medical waste.

In dentistry, autoclaves provide sterilization of dental instruments according to health technical memorandum 01-05 (HTM01-05).

According to HTM01-05, instruments can be kept, once sterilized using a vacuum autoclave for up to 12 months using sealed pouches.

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Fig 4.3 This is an autoclave that is used in the medical facility

Fig 4.4Other Autoclave with dental equipment in an autoclave to be sterilized for 2 hours at 150 to 180 degrees Celsius

Fig 4.5 Stovetop autoclaves—the simplest of autoclaves

Fig 4.6The machine on the right is an autoclave used for processing substantial quantities of laboratory equipment prior to reuse, and infectious material prior to disposal. (The machines on the left and in the middle are washing machines.)

Fig 4.7 The Autoclave must reach 121 degrees Celsius

The high temperatures cause the internal parts of the microorganisms to essentially cook. Once the internal parts cannot function in the microorganisms, they will die. The steam and pressure are released and brought down to normal room temperature and pressure after the 15

or more minutes of running. The items that were autoclaved will remain sterile until they are contaminated by new microorganisms.

EXPERIMENT NO- 4.3

***OBJECTIVE*:** Study of the principal and application of Elisa Reader

INTRODUCTION

ELISA stands for enzyme linked immune-sorbent assay. In short, it is an antibody test or a test for immune response to things attacking the body such as virus, bacteria and allergens. The test is done in an ELISA plate, also known as a 96-well plate or micro plate. The ELISA reader reads the plate.

WHAT AN ELISA READER DOES

An ELISA reader measures and quantities the color differences in the 12 wells of the plate.

ELISA readers or micro plate readers do spectrophotometer; they emit light at one wave length, and measure the amount of light absorbed and reflected by an object such as a protein. A spectrophotometer measures ultraviolet and visible light.

Additionally, ELISA plate readers can also measure fluorescence and luminescence. Chemical dyes fluoresce or emit one color or wavelength when exposed to light. The amount of reflection, absorption and the color identify, and measure the amount of a substance.

PURPOSE OF AN ELISA READER

ELISA readers were designed for measuring antibody tests. They worked so well, the machine has been adapted to other purposes. Researchers use them for protein and enzyme assays. They are also used for HIV detection and quantization of nucleic acids.

ADVANTAGES OF ELISA READER

Spectrophotometers require more sample per measurement .

To use a spectrophotometer or ELISA plate reader, the molecule must be dissolved in solution.

A spectrophotometer requires between 400 micro-liters and four milliliters, depending on the manufacturer and model. An ELISA plate reader needs about two to 100 micro-liters; ELISA plate readers use much less of a sample to get a result.

ELISA plate readers measure more samples in a shorter period of time. A spectrophotometer measures one to six samples at a time. Typically, an ELISA plate measures 96 wells in an equivalent amount of time.

Fig 4.8 Demonstration of ELISA reader

Fig 4.9 ELISA plate readers

EXPERIMENT NO- 4.4

***OBJECTIVE*:** Study of the principal and application of PCR Machine

INTRODUCTION

PCR (polymerase chain reaction) machine, also known as a thermal cycler, is a DNA amplifier that regulates temperature and amplifies segments of DNA via the polymerase chain reaction**.**

PCR machines are also sometimes used to facilitate reactions regarding enzyme digestion or rapid diagnostics.

PCR requires four main components. The first is the DNA sample containing the section, or sections, for copying.

Secondly, PCR requires a primer. Primers are short segments of DNA a scientist creates to match the DNA sample.

The next requirement of PCR is DNA polymerase, an enzyme that copies DNA. Human DNA polymerase denatures, or breaks down, at PCR temperatures, so researchers often use DNA polymerase from a heat-tolerant bacterium.

Finally, PCR requires nucleotides: adenine, guanine, cytosine and thymine. These are the base pairs that provide the coding elements of DNA.

Researchers first heat the PCR mixture to a temperature that denatures the DNA double helix.

A cooling step follows, which allows the primers to bind to the sample DNA. Another healing cycle follows during which DNA polymerase elongates the DNA strand.

Repeating these steps multiple times creates many new copies of the original DNA sample in as few as three hours.

PCR is useful in the diagnosis of viral diseases and some forms of cancer. It is also a common tool in forensic science for replicating small samples from crime scenes.

PCR is used for research when it is necessary to make a large amount of a single gene, such as for genetic engineering or cloning. PCR is also used to test whether or not a particular gene product is present in a sample.

In forensics, PCR technology is used to carry out DNA fingerprinting to analyze crime scene DNA evidence. PCR can also be used in medical settings to carry out tissue-typing for organ transplants.

Fig 4.10 PCR Machine

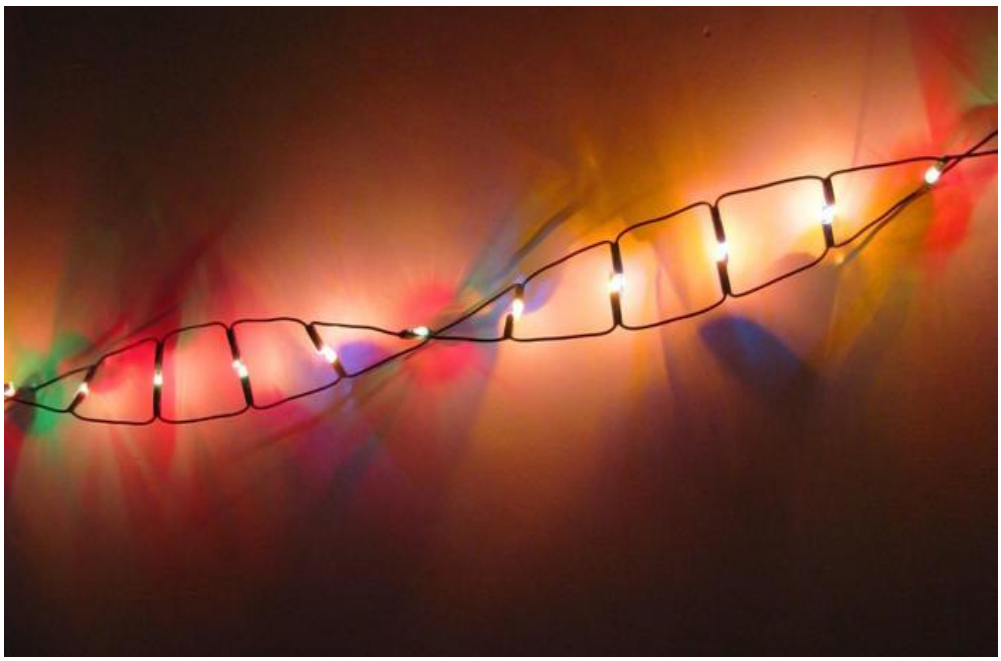
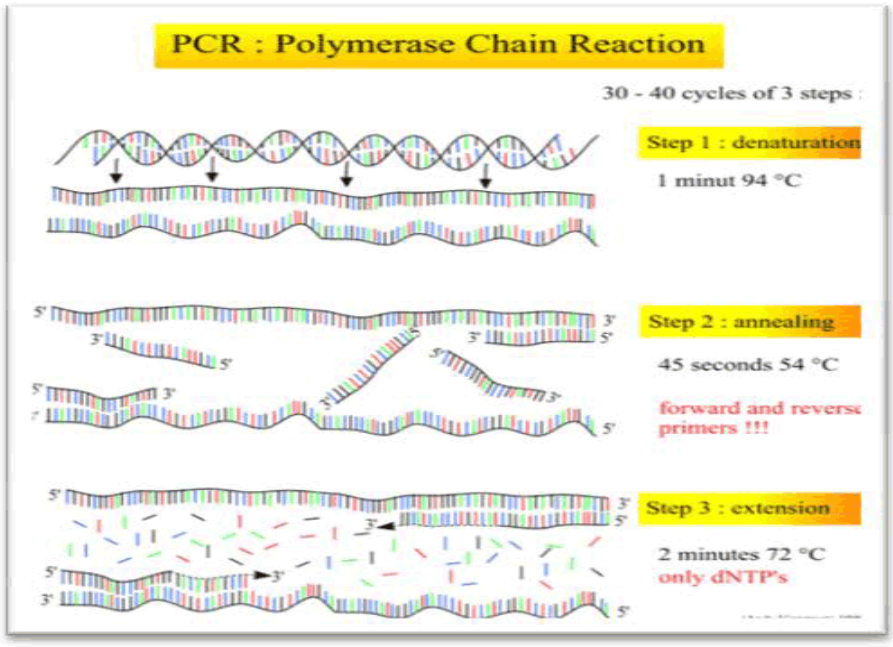


Fig 4.11The purpose of PCR is to amplify small amounts of a DNA sequence of interest so it can be analyzed separately. PCR can be used to make a large amount of a specific piece of DNA or to test a DNA sample for that sequence



Experiment No- 4.5

***OBJECTIVE*:** Study of the principal and application Refrigerated Centrifuge

INTRODUCTION

Refrigerated centrifuge works on the concept of sedimentation principle by holding up the sample tubes with a capacity of 2ml, 10ml and 50ml in rotation around a fixed axis.

In this, the centripetal force causes the denser substances to separate out along the radial direction in the bottom of the centrifuge tube.

The rate of the centrifugation is calculated by the acceleration applied to the sample and it is typically measured in revolution per minute (RPM) or relative centrifugal force (RCF).

The particle's settling velocity during centrifugation depends on the function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity.

This equipment is extensively used in chemistry, biology, and biochemistry for isolating and separating suspensions.

It additionally provides the cooling mechanism to maintain the uniform temperature throughout the operation of the sample.

Fig.4.12 Refrigerated centrifuge

Fig 4.13 Compact and quiet 4 x 85 ml refrigerated centrifuge for low-speed clinical and research applications

FUNCTIONS

- Fast Cool function eliminates long waits for set temperatures
- Easy-to-use interface with digital display improves repeatability

- Standby cooling maintains temperature when chamber is not in use
- -9 to 40°C temperature range

Centrifuge is ideal for low-speed spinning (up to 3,000 x g) of tubes ranging in volume from 1.1 to 85 ml.

The space-saving footprint and innovative design accommodates 30 x 15 ml round-bottom tubes or 20 x 15 ml conical tubes.

Automatic, motorized locking lid makes loading and closing effortless. A variety of rotors can be used for optimal versatility in high-capacity or sensitive applications.

For delicate blood and urine samples, users can deactivate the electronic brake.

Time and speed are easily adjusted and digitally displayed for accuracy and all settings are adjustable during a run.

Two preset buttons store routine runs for added convenience. Settings can also be locked to prevent unintentional adjustment.

Timer is programmable up to 99 minutes or continuous, and an audible alert signals the end of a run. Measures 15"W x 10.2"H x 22.8"D (38 x 26 x 58 cm). Weight 79 lbs./36 kg.

Centrifuge 5702R (refrigerated) with 4x100mL swing-bucket rotor (A-4-38) including buckets. Max. RCF 3,000xg (4400rpm),

Temperature range -9 - 40°C. Compact footprint, Whisper quiet operation, SOFT brake option, 120V/50-60Hz.

Centrifuge 5702R (refrigerated) with 4x100mL swing-bucket rotor (A-4-38) including buckets. Max. RCF 3,000xg (4400rpm),

EXPERIMENT NO- 4.6

***OBJECTIVE*:** Study of the principal and application of Tran illuminator

INTRODUCTION

Tran illuminators are used in molecular biology labs to view DNA (or RNA) that has been separated by electrophoresis through an agarose gel.

During or immediately after electrophoresis, the agarose gel is stained with a fluorescent dye which binds to nucleic acid.

Exposing the stained gel to a UVB light source causes the DNA/dye to fluoresce and become visible.

This technique is used wherever the researcher needs to be able to view their sample, for example sizing a PCR product, purifying DNA segment after a restriction enzyme digest, quantifying DNA or verifying RNA integrity after extraction.

It describes how to make a UVB (310nm) transilluminator with a 7 x 7 cm window for viewing ethidium bromide (or SYBR-Safe) stained DNA mini-gels.

Once all of the materials are collected, the actual assembly time is approx. 1-2 hours. Some soldering is required.

For the UV transilluminator enclosure and lid. Laser cut the parts from the material listed in the design file.

If you do not have access to a laser cutter, you can send the files to any laser cutting service such as Pololu. Materials for laser cutting can be found at any supplier of acrylic materials (McMaster-Carr, US Plastics etc) except for the solacryl (UV-transmissive) which can be bought from Loop Acrylics.

Tap holes in the following parts:

- 5-40: Two holes in the enclosure side with the cutout for the power switch
- 8-32: Four holes in the solacryl cover
- 8-32: Four holes in the 0.25" clear lid side part for mounting the hinges
- 8-32: Two holes in the enclosure bottom

Safety Notes:

1. Because ethidium bromide is a toxic chemical with strict safety protocols, it is only recommended that you use this dye in a lab with established handling, storage and waste disposal procedures in place**.** Other users are strongly recommended to use SYBR-Safe instead, which can be handled and disposed of more safely.
2. The transilluminator does come with a safety lid for viewing the gel. However, when the lid is not in place, safety glasses mustbe worn when operating the UVB bulb.
3. If you prefer to avoid UVB altogether, we can recommend the blue light LED transilluminators such as the one described in this instructable instead.

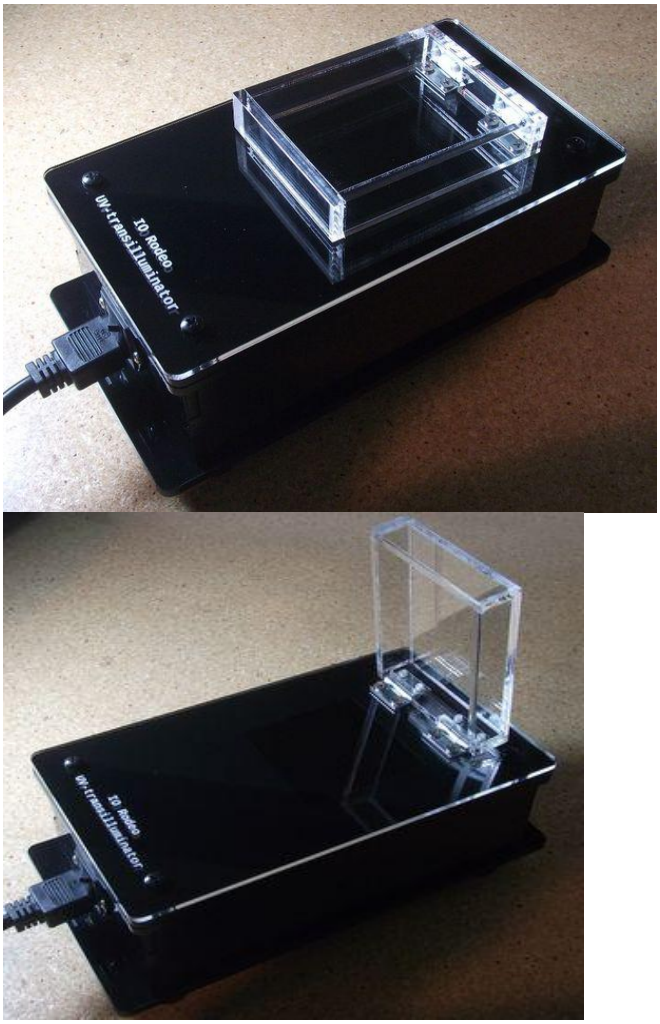


Fig.4.14 ***UV****-TransiIlluminator*

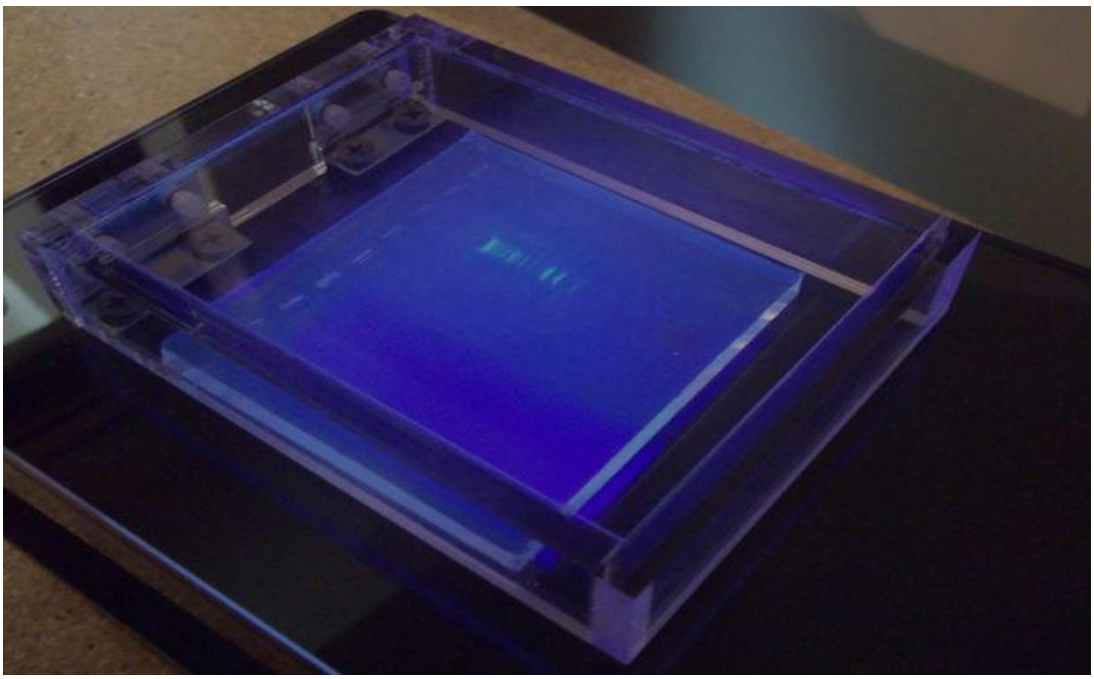


Fig 4.15 UV- Tran illuminator

EXPERIMENT NO- 02

***OBJECTIVE*: Study of the Double helical DNA model**

INTRODUCTION

Many people believe that American biologist James Watson and English physicist Francis Crick discovered DNA in the 1950s.

In reality, this is not the case. Rather, DNA was first identified in the late 1860s by Swiss chemist Friedrich Miescher.

Then, in the decades following Miescher's discovery, other scientists--notably, Phoebus Levene and Erwin Chargaff--carried out a series of research efforts that revealed additional details about the DNA molecule, including its primary chemical components and the ways in which they joined with one another.

Without the scientific foundation provided by these pioneers, Watson and Crick may never have reached their groundbreaking conclusion of 1953: that the DNA molecule exists in the form of a three-dimensional double helix.

Watson and Crick Propose the Double Helix

Chargaff's realization that A = T and C = G, combined with some crucially important X-ray crystallography work by English researchers Rosalind Franklin and Maurice Wilkins, contributed to Watson and Crick's derivation of the three-dimensional, double-helical model for the structure of DNA.

Watson and Crick's discovery was also made possible by recent advances in model building, or the assembly of possible three-dimensional structures based upon known molecular distances and bond angles, a technique advanced by American biochemist Linus Pauling.

In fact, Watson and Crick were worried that they would be "scooped" by Pauling, who proposed a different model for the three-dimensional structure of DNA just months before they did. In the end, however, Pauling's prediction was incorrect.

Using cardboard cutouts representing the individual chemical components of the four bases and other nucleotide subunits, Watson and Crick shifted molecules around on their desktops, as though putting together a puzzle.

They were misled for a while by an erroneous understanding of how the different elements in thymine and guanine (specifically, the carbon, nitrogen, hydrogen, and oxygen rings) were configured.

Only upon the suggestion of American scientist Jerry Donohue did Watson decide to make new cardboard cutouts of the two bases, to see if perhaps a different atomic configuration would make a difference. It did. Not only did the complementary bases now fit together perfectly (i.e., A with T and C with G), with each pair held together by hydrogen bonds, but the structure also reflected Chargaff's rule.

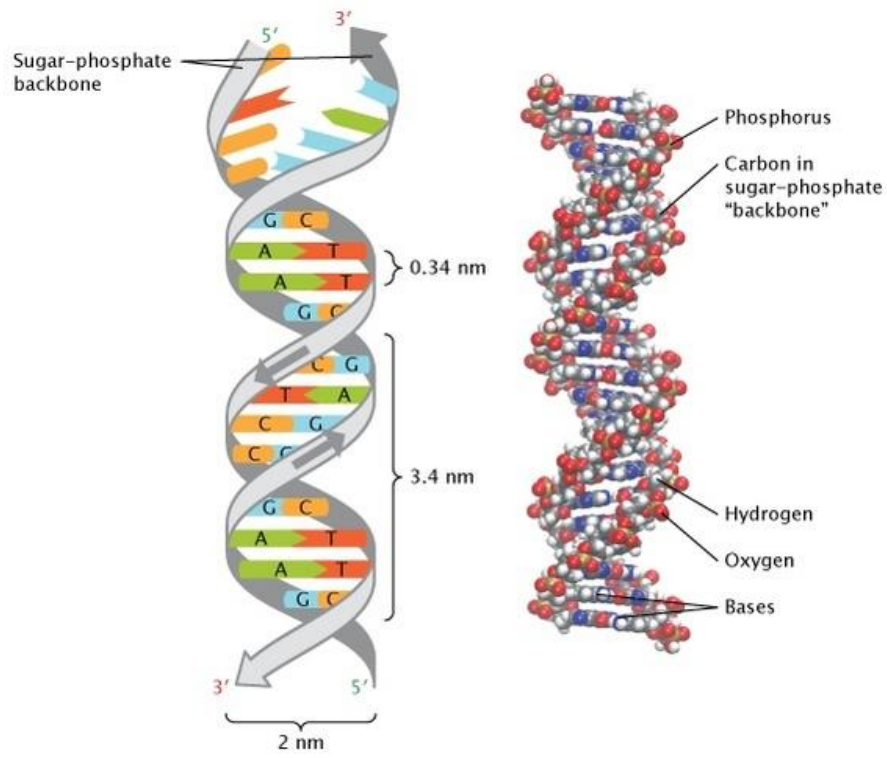


Fig 4.16 The double-helical structure of DNA,The 3- dimensional double helix structure of DNA, correctly elucidated by James Watson and Francis Crick. Complementary bases are held together as a pair by hydrogen bonds.

Although scientists have made some minor changes to the Watson and Crick model, or have elaborated upon it, since its inception in 1953, the model's four major features remain the same yet today. These features are as follows:

- DNA is a double-stranded helix, with the two strands connected by hydrogen bonds.
- A base is always paired with Ts, and Cs are always paired with Gs, which is consistent with and accounts for Chargaff's rule.
- Most DNA double helices are right-handed; that is, if you were to hold your right hand out, with your thumb pointed up and your fingers curled around your thumb, your thumb would represent the axis of the helix and your fingers would represent the sugar-phosphate backbone. Only one type of DNA, called Z-DNA, is left-handed.
- The DNA double helix is anti-parallel, which means that the 5' end of one strand is paired with the 3' end of its complementary strand (and vice versa).

- As shown in Figure 4, nucleotides are linked to each other by their phosphate groups, which bind the 3' end of one sugar to the 5' end of the next sugar.
- Not only are the DNA base pairs connected via hydrogen bonding, but the outer edges of the nitrogen-containing bases are exposed and available for potential hydrogen bonding as well.
- These hydrogen bonds provide easy access to the DNA for other molecules, including the proteins that play vital roles in the replication and expression of DNA.

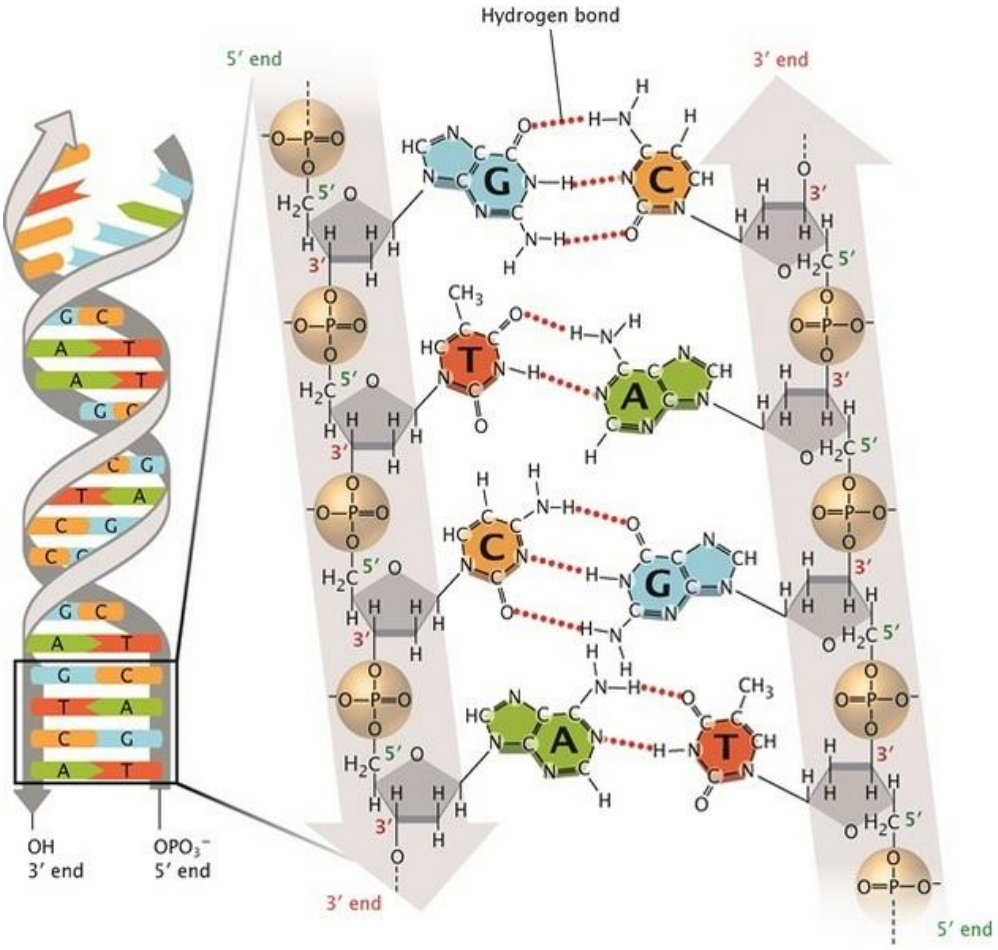


Fig 4.17 Base pairing in DNA

Two hydrogen bonds connect T to A; three hydrogen bonds connect G to C. The sugar-phosphate backbones (grey) run anti-parallel to each other, so that the 3' and 5' ends of the two strands are aligned.

One of the ways that scientists have elaborated on Watson and Crick's model is through the identification of three different conformations of the DNA double helix.

In other words, the precise geometries and dimensions of the double helix can vary. The most common conformation in most living cells (which is the one depicted in most diagrams of the double helix, and the one proposed by Watson and Crick) is known as B-DNA.

There are also two other conformations: A-DNA, a shorter and wider form that has been found in dehydrated samples of DNA and rarely under normal physiological circumstances; and Z-DNA, a left-handed conformation. Z-DNA is a transient form of DNA, only occasionally existing in response to certain types of biological activity.

Z-DNA was first discovered in 1979, but its existence was largely ignored until recently. Scientists have since discovered that certain proteins bind very strongly to Z-DNA, suggesting that Z-DNA plays an important biological role in protection against viral disease (Rich & Zhang, 2003).

Three different conformations of the DNA double helix

(A) A-DNA is a short, wide, right-handed helix. (B) B-DNA, the structure proposed by Watson and Crick, is the most common conformation in most living cells. (C) Z-DNA, unlike A- and B-DNA, is a left-handed helix.

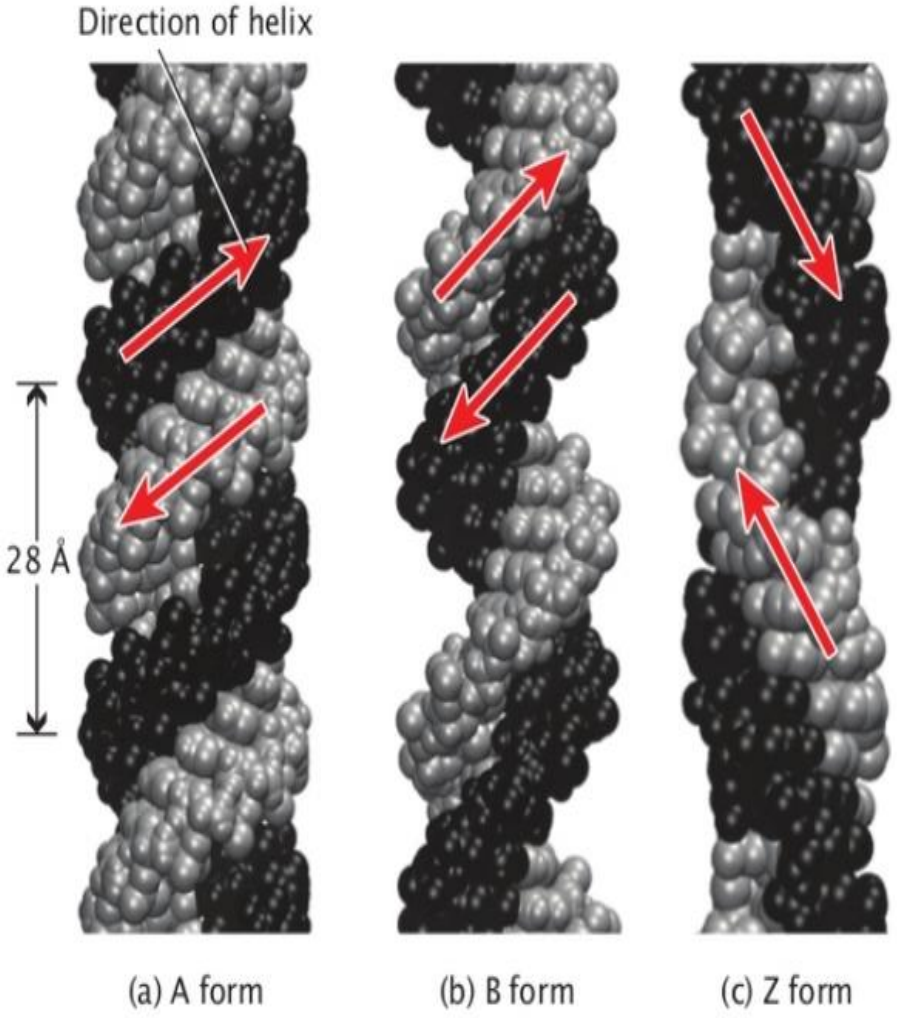
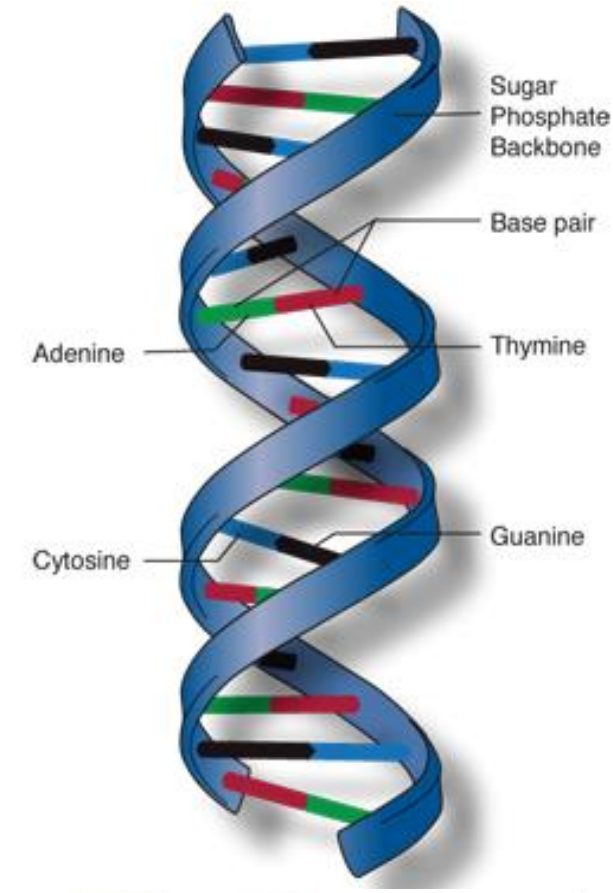


Fig.4.18 Three different conformations of the DNA double helix



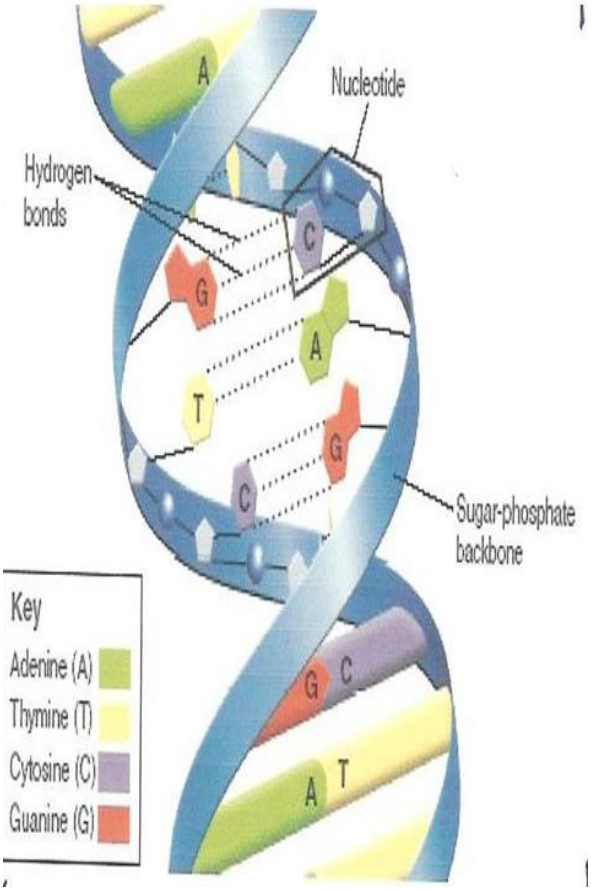


Fig.4.19 double Helical structure of DNA

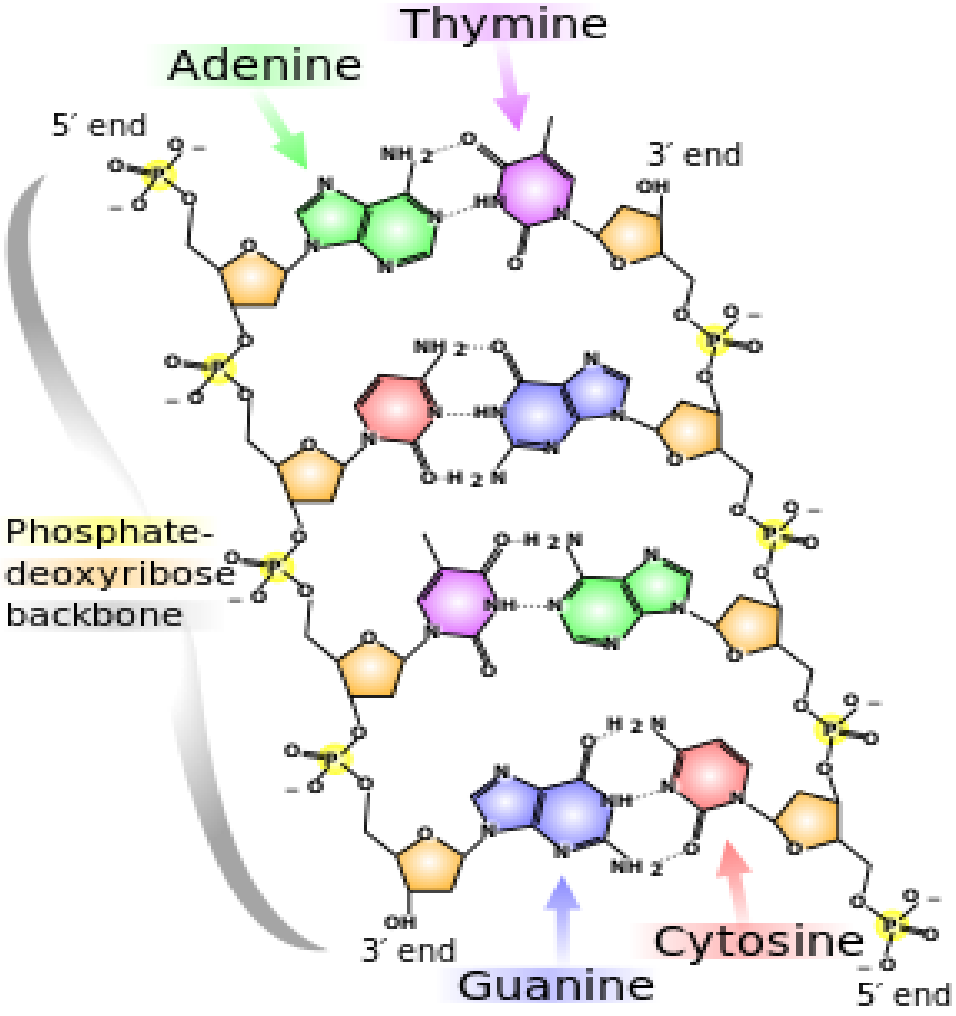

Thymine
Adenine
5′ end
3′ end
Phosphate-
deoxyribose
backbone
3′ end
Guanine
Cytosine
5′ end

Fig.4.20 Chemical structure of DNA; hydrogen bonds shown as dotted lines

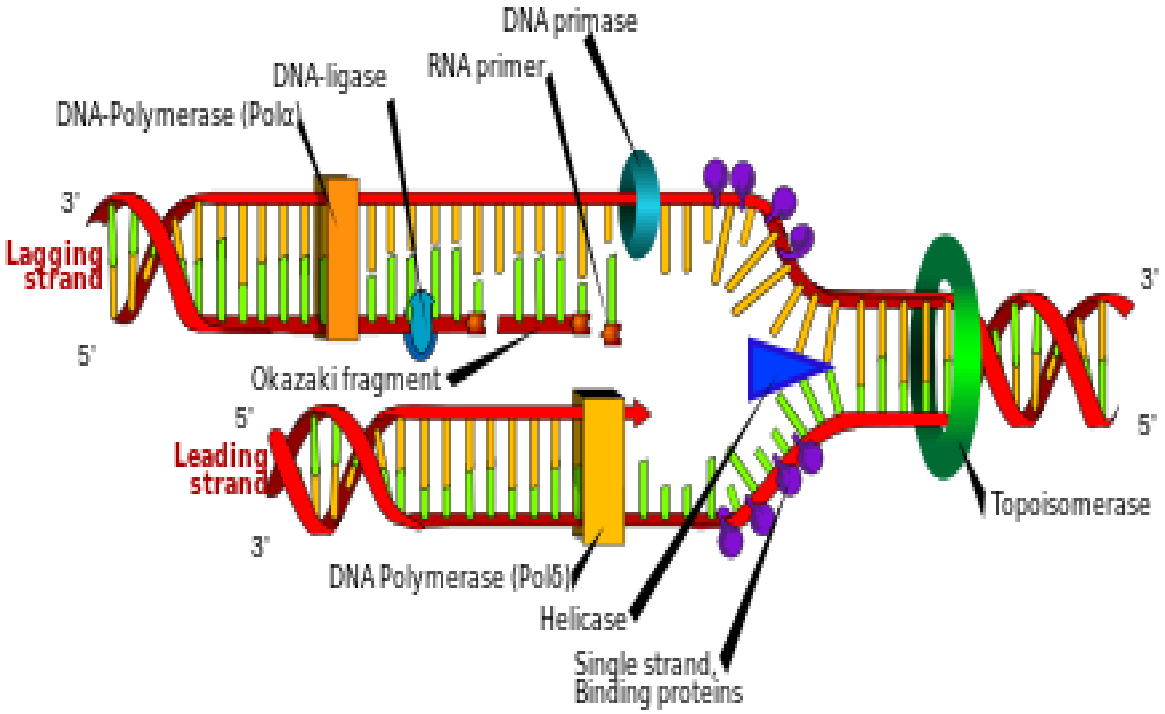


Fig.4.21 DNA replication

(The double helix is unwound by a helicase and topoisomerase. Next, one DNA polymerase produces the leading strand copy. Another DNA polymerase binds to the lagging strand. This enzyme makes discontinuous segments (called Okazaki fragments) before DNA ligase joins them together.)

EXPERIMENT NO- 03

OBJECTIVE: Study of the Chromatography or Thin layer Chromatography (TLC)

INTRODUCTION

Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose.

This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

The mobile phase has different properties from the stationary phase.

For example, with silica gel, a very polar substance, non-polar mobile phases such as heptanes are used.

The mobile phase may be a mixture, allowing chemists to fine-tune the bulk properties of the mobile phase.

After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light onto the sheet; the sheets are treated with a phosphor, and dark spots appear on the sheet where compounds absorb the light impinging on a certain area.

Chemical processes can also be used to visualize spots; anisaldehyde, for example, forms colored adducts with many compounds, and sulfuric acid will char most organic compounds, leaving a dark spot on the sheet.

To quantify the results, the distance traveled by the substance being considered is divided by the total distance traveled by the mobile phase. (The mobile phase must not be allowed to reach the end of the stationary phase.)

This ratio is called the retention factor or R_f. In general, a substance whose structure resembles the stationary phase will have low R_f, while one that has a similar structure to the mobile phase will have high retention factor.

Retention factors are characteristic, but will change depending on the exact condition of the mobile and stationary phase. For this reason, chemists usually apply a sample of a known compound to the sheet before running the experiment.

Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance.

Specific examples of these applications include: analyzing ceramides and fatty acids, detection of pesticides or insecticides in food and water, analyzing the dye composition of fibers in forensics, assaying the radiochemical purity of radiopharmaceuticals, or identification of medicinal plants and their constituents

A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantitative analysis.

This method is referred to as HPTLC, or "high-performance TLC". HPTLC typically uses thinner layers of stationary phase and smaller sample volumes, thus reducing the loss of resolution due to diffusion.

Fig.4.22 Separation of black ink on a TLC plate

TLC of three standards (ortho-, meta- and para-isomers) and a sample

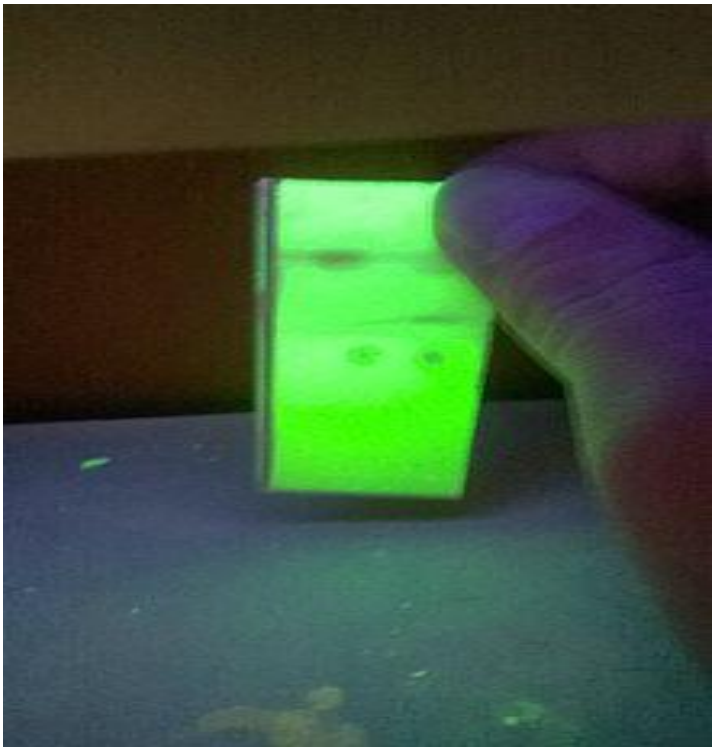


Fig.4.23 Fluorescent TLC plate under UV light

PLATE PREPARATION

TLC plates are usually commercially available, with standard particle size ranges to improve reproducibility.

They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water.

This mixture is spread as thick slurry on an uncreative carrier sheet, usually glass, thick aluminum foil, or plastic.

The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the absorbent layer is typically around 0.1 – 0.25 mm for analytical purposes and around 0.5 – 2.0 mm for preparative TLC.

TECHNIQUE

The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases.

Because of its simplicity and speed TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products.

Plates can be labeled before or after the chromatography process using a pencil or other implement that will not interfere or react with the process.

To run a thin layer chromatography plate, the following procedure is carried out:

Using a capillary, a small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge.

The solvent is allowed to completely evaporate off to prevent it from interfering with sample's interactions with the mobile phase in the next step. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber.

This step is often repeated to ensure there is enough analyte at the starting spot on the plate to obtain a visible result. Different samples can be placed in a row of spots the same distance from the bottom edge, each of which will move in its own adjacent lane from its own starting point.

- A small amount of an appropriate solvent (eluent) is poured into a glass beaker or any other suitable transparent container (separation chamber) to a depth of less than 1 centimeter.
- A strip of filter paper (aka "wick") is put into the chamber so that its bottom touches the solvent and the paper lies on the chamber wall and reaches almost to the top of the container. The container is closed with a cover glass or any other lid and is left for a few minutes to let the solvent vapors ascend the filter paper and saturate the air in the chamber. (Failure to saturate the chamber will result in poor separation and non-reproducible results).
- The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample).

- The plate should be removed from the chamber before the solvent front reaches the top of the stationary phase (continuation of the elution will give a misleading result) and dried.
- Without delay, the solvent front, the furthest extent of solvent up the plate, is marked.
- The plate is visualized. As some plates are pre-coated with a phosphor such as zinc sulfide, allowing many compounds to be visualized by using ultraviolet light; dark spots appear where the compounds block the UV light from striking the plate. Alternatively, plates can be sprayed or immersed in chemicals after elution. Various visualising agents react with the spots to produce visible results.

SEPARATION PROCESS AND PRINCIPLE

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase and because of differences in solubility in the solvent.

By changing the solvent, or perhaps using a mixture, the separation of components (measured by the Rf value) can be adjusted. Also, the separation achieved with a TLC plate can be used to estimate the separation of a flash chromatography column. (A compound elutes from a column when the amount of solvent collected is equal to 1/Rf.) Chemists often use TLC to develop a protocol for separation by chromatography and they use TLC to determine which fractions contain the desired compounds.

Analysis

As the chemicals being separated may be colorless, several methods exist to visualize the spots:

- Fluorescent analytes like quinine may be detected under black light (366 nm)
- Often a small amount of a fluorescent compound, usually manganese-activated zinc silicate, is added to the adsorbent that allows the visualization of spots under UV-C light (254 nm). The adsorbent layer will thus fluoresce light-green by itself, but spots of analyte quench this fluorescence.
- Iodine vapors are a general unspecific color reagent
- Specific color reagents into which the TLC plate is dipped or which are sprayed onto the plate exist.

    - Potassium permanganate - oxidation
    - Bromine
- In the case of lipids, the chromatogram may be transferred to a PVDF membrane and then subjected to further analysis, for example mass spectrometry, a technique known as Far-Eastern blotting.

Once visible, the R_f value, or retardation factor, of each spot can be determined by dividing the distance the product traveled by the distance the solvent front traveled using the initial spotting site as reference. These values depend on the solvent used and the type of TLC plate and are not physical constants.

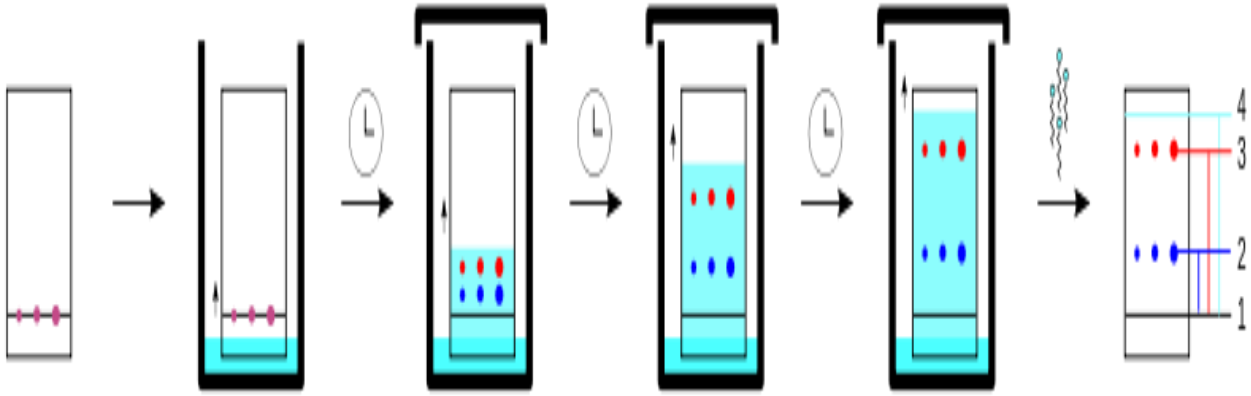


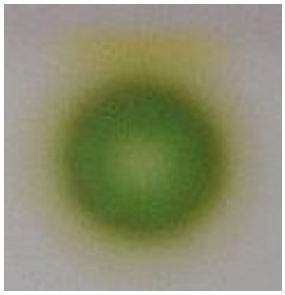
Fig.4.24 Development of a TLC plate, a purple spot separates into a red and blue spot

ISOLATION

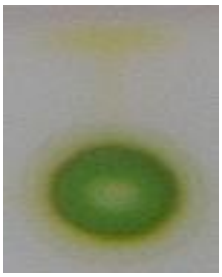
Since different compounds will travel a different distance in the stationary phase, chromatography can in effect be used as an isolation technique.

The separated compounds each occupying a specific area on the plate, they can be scraped away, put in another solvent to separate them from the stationary phase and used for further analysis.

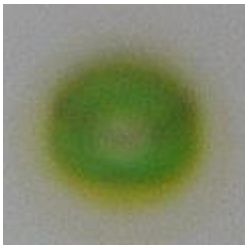
As an example, in the chromatography of an extract of green leaves (for example spinach) in 7 stages of development, Carotene elutes quickly and is only visible until step 2. Chlorophyll A and B are halfway in the final step and lutein the first compound staining yellow. Once the chromatography is over, the carotene can be removed from the plate, put back into a solvent and ran into a spectrophotometer to characterize its wavelength absorption.

- 

Step 1

- 

Step 2

- 

Step 3

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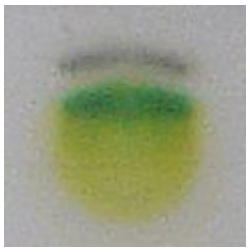
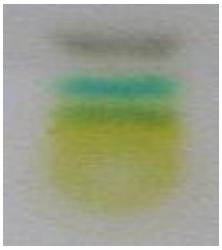
Step 4

- 

Step 5

- 

Step 6

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Step 7

Fig.4.25 Isolation

EXPERIMENT NO- 04

***OBJECTIVE*:** Study of the Recombinant DNA techniques

INTRODUCTION

Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure. They differ only in the nucleotide sequence within that identical overall structure.

Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands.

Recombinant DNA molecules are sometimes called chimeric DNA, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

The DNA sequences used in the construction of recombinant DNA molecules can originate from any species.

For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA.

In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules.

Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

Proteins that can result from the expression of recombinant DNA within living cells are termed recombinant proteins.

When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced.

Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences[1]

Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

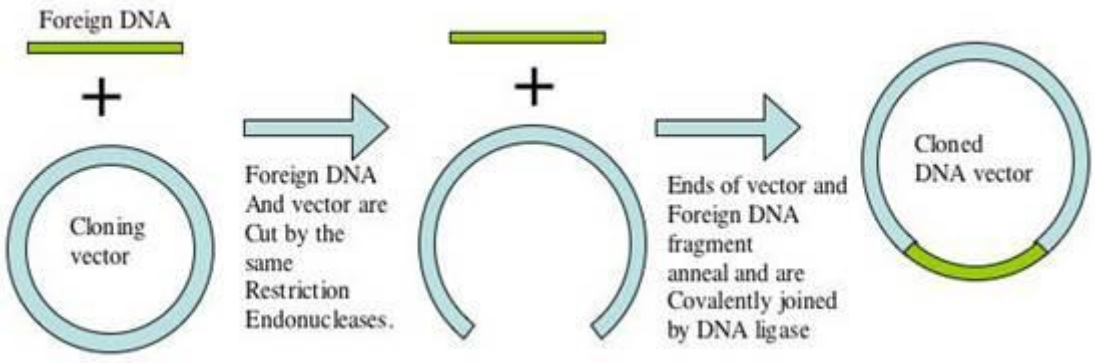

Fig.4.26 Construction of recombinant DNA, in which a foreign DNA fragment is inserted into a plasmid vector.In this example, the gene indicated by the white color is inactivated upon insertion of the foreign DNA fragment.

PROPERTIES OF ORGANISMS CONTAINING RECOMBINANT DNA

In most cases, organisms containing recombinant DNA have apparently normal phenotypes.

That is, their appearance, behavior and metabolism are usually unchanged, and the only way to demonstrate the presence of recombinant sequences is to examine the DNA itself, typically using a polymerase chain reaction (PCR) test. Significant exceptions exist, and are discussed below.

If the rDNA sequences encode a gene that is expressed, then the presence of RNA and/or protein products of the recombinant gene can be detected, typically using RT-PCR or western hybridization methods.

Gross phenotypic changes are not the norm, unless the recombinant gene has been chosen and modified so as to generate biological activity in the host organism.

Additional phenotypes that are encountered include toxicity to the host organism induced by the recombinant gene product, especially if it is over-expressed or expressed within inappropriate cells or tissues.

In some cases, recombinant DNA can have deleterious effects even if it is not expressed. One mechanism by which this happens is insertional inactivation, in which the rDNA becomes inserted into a host cell's gene.

In some cases, researchers use this phenomenon to "knock out" genes to determine their biological function and importance. Another mechanism by which rDNA insertion into chromosomal DNA can affect gene expression is by inappropriate activation of previously unexpressed host cell genes.

This can happen, for example, when a recombinant DNA fragment containing an active promoter becomes located next to a previously silent host cell gene, or when a host cell gene that functions to restrain gene expression undergoes insertional inactivation by recombinant DNA.

Uses:

Recombinant DNA is widely used in biotechnology, medicine and research. Today, recombinant proteins and other products that result from the use of DNA technology are

found in essentially every western pharmacy, doctors or veterinarian's office, medical testing laboratory, and biological research laboratory.

The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences.

Recombinant DNA is used to identify, map and sequence genes, and to determine their function. rDNA probes are employed in analyzing gene expression within individual cells, and throughout the tissues of whole organisms.

Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms.

Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture, and bioengineering. Some specific examples are identified below.

Recombinant chymosin: Found in rennet, chymosin is an enzyme required to manufacture cheese.

Recombinant human insulin: Almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of insulin-dependent diabetes.

Recombinant human growth hormone (HGH, somatotropin): Administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development.

Recombinant blood clotting factor VIII: A blood-clotting protein that is administered to patients with forms of the bleeding disorder hemophilia, who are unable to produce factor VIII in quantities sufficient to support normal blood coagulation.

Recombinant hepatitis B vaccine: Hepatitis B infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells.

Diagnosis of infection with HIV: Each of the three widely used methods for diagnosing HIV infection has been developed using recombinant DNA.

Golden rice: A recombinant variety of rice that has been engineered to express the enzymes responsible for β-carotene biosynthesis.

Herbicide-resistant crops: Commercial varieties of important agricultural crops (including soy, maize/corn, sorghum, canola, alfalfa and cotton) have been developed that incorporate a recombinant gene that results in resistance to the herbicide glyphosate (trade name *Roundup*), and simplifies weed control by glyphosate application.

Insect-resistant crops: *Bacillus thuringeiensis* is a bacterium that naturally produces a protein (Bt toxin) with insecticidal properties. The bacterium has been applied to crops as an insect-control strategy for many years, and this practice has been widely adopted in agriculture and gardening.

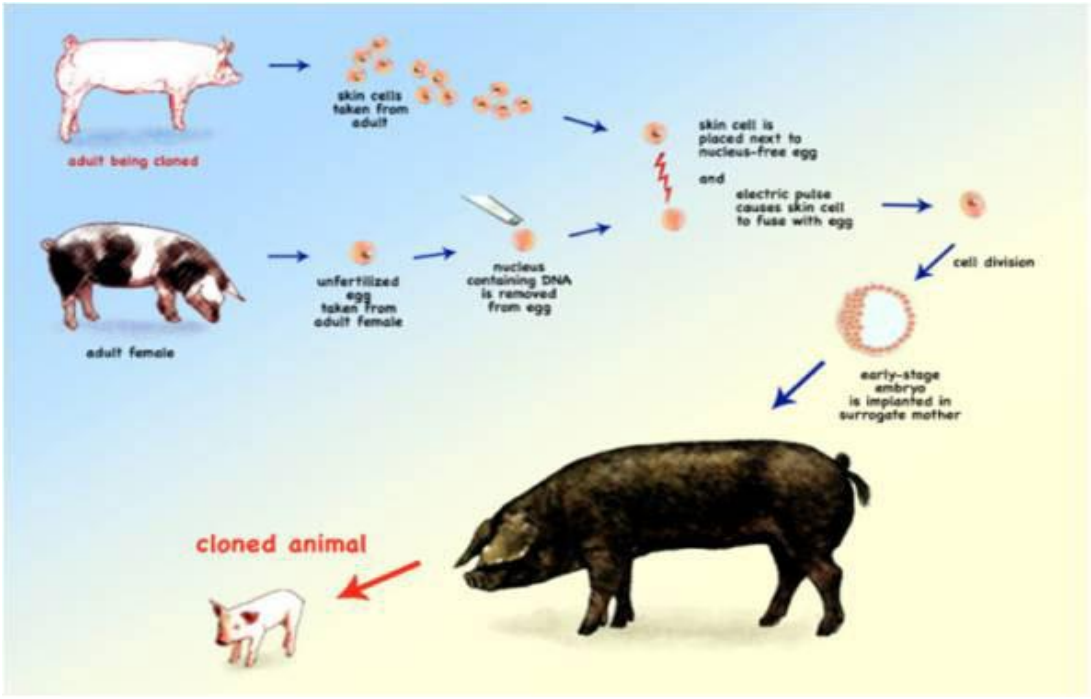

Fig.4.26 Diagram of pigs to show how animal cloning is carried out

SUMMERY

1. Laminar Flow Layers of water flow over one another at different speeds with virtually no mixing between layers.

2. The flow velocity profile for laminar flow in circular pipes is parabolic in shape, with a maximum flow in the center of the pipe and a minimum flow at the pipe walls. The average flow velocity is approximately one half of the maximum velocity.

Turbulent Flow The flow is characterized by the irregular movement of particles of the fluid.

4. Thanks to researchers such as these, we now know a great deal about genetic structure, and we continue to make great strides in understanding the human genome and the importance of DNA to life and health.

5. PCR (polymerase chain reaction) denotes a process that is used to replicate DNA.

6. The first step in PCR, known as denaturing, involves heating a DNA sample to separate its two strands. Once separated the two strands are used as templates to synthesise two new DNA strands. This is done with the help of an enzyme called Taq polymerase.

Recombinant DNA is an artificial form of DNA that cannot be found in natural organisms. It is made in the laboratory by joining together genes taken from different sources. This is done by selecting and cutting out a gene at a specific point on a strand of DNA using restriction enzymes which act as molecular scissors.

8. Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose.

GLOSSARY

1. **Biotechnology:** A general term used to describe the use of biological processes to make products, in contrast to purely chemical processes. Biotechnology has been in practice for centuries and includes such traditional applications as the use of yeast in making beer, as well as modern applications like recombinant DNA techniques to improve crops.

2. **Recombinant DNA:** The DNA formed when DNA fragments from more than one organism are spliced together in vitro.

3. **Sequencing of DNA Molecules:** The process of finding the order of nucleotides (guanine, adenine, cytosine and thymine) that make up a DNA or RNA fragment.

4. **Stem cell :** A fundamental cell that has the potential to develop into any of the 210 different cell types found in the human body. Human life begins with stem cells, which divide again and again and branch off into special roles, like becoming liver or heart cells.

They are an important resource for disease research and for the development of new ways to treat disease.

5. Transcription: A process in the cell where the DNA is used as a template to make the messenger RNA.

6. **Reynolds number:** Fluid flowing at a lower rate with elements of the fluid flowing in fixed streamlines. Laminar friction is a function of N_{Re} (Reynolds number). For laminar flow, the Fanning fracition factor = $16/N_{Re}$.

7**. Assay:** A method for determining the presence or quantity of a component.

8. **Clone:** A genetically identical copy of an organism or of a specific piece of DNA for use in research.

9. **Genetic mapping:** A research method that collects genetic information to determine the relative position of a gene or a phenotype in the genome.

10. Microarray: A glass or plastic slide with many DNA spots attached to it, which allows researchers to study how many genes act and interact in different conditions.

11. Plasmid: A DNA structure that is separate from the cell's genome and can replicate independently of the host cell. Plasmids are used in the laboratory to deliver specific DNA sequences into a cell.

SELF ASSESSMENT QUESTIONS

1. How biotechnology useful to human beings and animals?
2. What will be the use of recombinant DNA technology?
3. What is base pair?
4. What is genetic engineering?
5. How DNA sequencing completed?

REFERENCES

- Rosano, Germán L.; Ceccarelli, Eduardo A. (2014-04-17). "Recombinant protein expression in Escherichia coli: advances and challenges". Frontiers in Microbiology.

- Hannig, G.; Makrides, S. (1998). "Strategies for optimizing heterologous protein expression in Escherichia coli". Trends in Biotechnology. 16 (2): 54–60. doi:10.1016/S0167-7799(97)01155-4. PMID 9487731.
- Brown, Terry (2006). Gene Cloning and DNA Analysis: an Introduction. Cambridge, MA: Blackwell Pub. ISBN 1-4051-1121-6.
- Dame RT (May 2005). "The role of nucleoid-associated proteins in the organization and compaction of bacterial chromatin". Molecular Microbiolo.
- Biocompare (2016). Microplate readers. Retrieved from January 22, 2015. http://www.biocompare.com/Lab-Equipment/20131-Microplate-Reader-ELISA-Plate-Reader/
- Susan R. Mikkelsen & Eduardo Cortón. Bioanalytical Chemistry, Ch. 13. Centrifugation Methods. John Wiley & Sons, Mar 4, 2004, pp. 247-267.
- Harry W. Lewis & Christopher J. Moody (13 Jun 1989). Experimental Organic Chemistry: Principles and Practice (Illustrated ed.). WileyBlackwell. pp. 159–173. ISBN 978-0-632-02017-1.
- Reich, E.; Schibli A. (2007). High-performance thin-layer chromatography for the analysis of medicinal plants (Illustrated ed.). New York: Thieme. ISBN 3-13-141601-7.

SUGGESTED READINGS

a. Biotechnology: Principles and Applications: H.K Das.
b. Biotechnology: A Problem Approach: Pranav Kumar and Usha Mina.
c. Animal Cell Culture and Technology (THE BASICS (Garland Science) by Michael Butler
d. A Text Book of Biotechnology – R.C. Dubey

TERMINAL QUESTIONS

1. Elaborate DNA recombinant technology?

2. Describe general steps of gene cloning.

3. Discuss method, principle of thin layer chromatography.

4. Who gave the DNA double helix model discuss in details.

5. Write the use of Tran illuminators in details.