Introduction to Microbiology

The word 'Microbiology' is derived from two Greek word Mikros = small and Bios = life. It is defined as a branch of science which involves the study of various micro organisms. It deals with morphology, taxonomy, physiology, genetics, cultures, pathophysiology and applications of micro organisms. Microbiology is broadly classified into two branches – Pure microbiology and Applied microbiology.

- Pure microbiology is again divided into various sub divisions - Bacteriology (study of bacteria), Mycology (study of fungi), Protozoology (study of protozoa), Phycology (study of algae), Parasitology (study of parasites), Immunology (study of the immune system), Microbial cytology (study of cellular structures, microscopic and submicroscopic details of microorganisms), Microbial physiology (study of physiological characters of microorganisms), Microbial ecology (study of relationship of microorganisms in the ecological systems), and Microbial genetics (study of genetic organisation of the micro organisms). Virology (study of viruses) is also a part of pure microbiology.

- Applied microbiology is a branch of microbiology in which the concepts of microbiology are applied to various fields. It is subdivided into – Medical microbiology (study of disease causing microorganisms - pathogen microbes), Industrial microbiology (study of micro organisms that are applied in industries), Biotechnology (study of microorganisms applied in the manufacture of drugs by genetic manipulation of micro organisms), Food microbiology (study of microorganisms applied in the manufacture of food products), Agriculture microbiology (study of role of microorganisms in the agricultural field), Environmental microbiology (study of role of microorganisms in the environment), Veterinary microbiology (study of micro organisms in veterinary science), Water microbiology or Aquatic microbiology(study of microorganisms that are found in water), Aero microbiology or Air microbiology(study of airborne microorganisms) and Pharmaceutical microbiology (study of micro organisms that are applied in the manufacturing of various pharmaceutical drugs).

Major Divisions of Microbial World

To further understand the microbes, it is very important to understand that initially all living organisms were classified into two categories plant kingdom and animal kingdom. Due to the presence of cell wall the bacteria was placed into the plant kingdom. Later on Ernst Haeckel in 1866 introduced a new kingdom called Protista and placed all the unicellular organisms in this kingdom. The unicellular protists are again divided as higher protistas – the living organisms that resemble like plants or animals for e.g. fungi, algae, and protozoa, and lower protists like bacteria, cyanobacteria and arche. In 1960, R.H. Whittaker suggested a five kingdom classification which includes – Kingdom Monera (bacteria, cyanobacteria, and archaea), Kingdom Protista (protozoa, algae), Kingdom Fungi, Kingdom Plantae, and Kingdom Animalia.

All the microorganisms are placed under three kingdoms i.e. Kingdom Monera, Kingdom Protista, and Kingdom Fungi.

	Monera	Protista	Fungi
Type	Prokaryote	Eukaryote	Eukaryote
Nutrition	absorption, chemosynthesis, photoheterotrophic/ photoautotrophic	absorption, photoautotrophic, and ingestion	Heterotopic, absorption, saprobic, and parasitic.
Metabolism	Aerobic, anaerobic, facultative anaerobic and microaerophilic	Usually aerobic	Aerobic
Reproduction	Asexual reproduction i.e. binary fission	Asexual, truesexual (plasmogamy, karyogamy)	Asexual and sexual
Motility	motile (flagella) and non-motile	Non-motile, motile - flagella, cilia, pseudopodia	Usually non-motile
Cellular Organization	Unicellular, solitary, colonial forms, filamentous, mycelial	Unicellular, multicellular, complex cell functions	Unicellular (Yeast) and multicellular
Examples	Bacteria, Cyanobacteria and Archae bacteria	Protozoa, Algae	Fungi

There was significant criticism from some biologists about the five kingdom classification mainly because the archaea show similarity with the eukaryotes with their genetical consideration. Due to this Woese et al in 1977 proposed a three domain system which is biological classification that divides cellular forms based on 16S rRNA homology into three: archaea, bacteria (eubacteria and cyanobacteria) and eukaryote. Later a six kingdom classification was introduced which includes, archaea, bacteria (prokaryotes) protista, fungi, plantae, and animalia (eukaryotes) which developed into a even kingdom classification later on which proposes protista to be divided into protozoa and chromysta.

Relationship Between Microbial Divisions

The relationship between microbial divisions can be studied using – The phylogenetic tree or evolutionary tree in which the 16S rRNA homology is used. The Phylogenetic tree showing the inferred evolutionary relationships is a branching diagram or tree that highlights the similarities and differences in the physical or genetic characteristics of various biological species.The phylogenetic tree can be divided into two main types – Rooted tree and unrooted tree. In rooted tree, the taxa are joined together and has common ancestors. The common point from which the branching starts is called a node. Each node is referred to taxonomic unit. The unrooted tree is where the taxa are joined together where common ancestors are unknown.

A rooted phylogenetic tree is a rooted tree with a unique node corresponding to the recent common ancestor. For example in a rooted phylogenetic tree where the starting point/common point node is LUCA (Last Universal Common Ancestor) three domains arise as branches i.e, bacteria, archaea and eukaryotes. From the domain bacteria various nodes and evolutionary branches like gram positive bacteria, green filamentous bacteria, spirochetes, proteobacteria etc. arise. The domains archae and eukaryotes have a common node which means that they have the common ancestors i.e. archae have much similarities with that of eukaryotes. The archae and eukaryotes are again bifurcated into different sub-branches

Methods of Classification of Microbes

Universally, all the organisms are classified into various groups based on the taxonomical hierarchy. According to this all the organisms are divided in the following schematic way: Domain → Kingdom → Phylum → Class → Order → Family → Genus → Species. Nomenclature is the naming system in which a particular name is given to each organism that is universally accepted. The commonly used nomenclature in the classification is a binomial nomenclature i.e. two words are used, in which the first word indicates the Genus of the organism and the second word indicates the Species of the organism. The name should be written in Italics. The generic name should start with capital letter and the species name should start with small letter. For example: Escherichia coli is a bacteria in which 'Escherichia' is the generic name and 'coli' is the species name.

Study of Bacteria

Bacteria are microscopic prokaryotic unicellular organisms.The size of bacteria is about 1.0 – 3.0 µm in length and 0.5 - 1.5 µm in diameter.

Classification of Bacteria according to shapes of Bacteria or Morphology:The bacteria exists in a wide diversity of shape and sizes. Bacterial species are either spherical shaped bacteria are called cocci(Greek: grain,seed) or rod shaped called bacilli (Latin:Stick). There are other shaped bacteria which are helically curved called spirilla and spirochetes, pleomorphic shaped (bacteria changes its shape according to the environment) and other shapes like pear shape, lobe, coma shape and disc shape etc.,

The type of classification of Bacteria according to spherical shape(cocci) is as follows

1. Monococci- single cocci:they are also called micrococcus and represented by single, discrete round cell.
2. Diplococci-two cocci: the cells remain attached to each other
3. Streptococci- more than two cocci:the cells are arranged in the form of chain
4. Tetracocci: four cocci forms are arranged in square like manner
5. Staphylococci:bunch of cocci forms are attached to one another
6. Tetrad shaped:cocci arranged in squares of 4
7. Sarcina shaped: cocci arranged in cubes of 8

The type of classification of Bacteria according to rod shape(bacilli) is as follows

1. Monobacilli: Bacilli appear as single rods
2. Diplobacilli: Diplobacilli appear in pairs after division.
3. Streptobacilli:The bacilli are arranged in chains, as the cells divide in one plane.
4. Coccibacilli: The bacilli shaped like coccus are short in nature.
5. Palisade Bacilli: The bacilli are attached at different angle and have angular patterns resulting due to bacilli bend at the points of attachment.
6. Mycelial or filamentous bacilli (actinomycetes): The filaments are branched, aseptate and thin.

The external structure of bacteria consists of flagella, pili or fimbriae, and capsule. The flagellum is a long helical hair like structure which helps in the movement of bacteria. The flagellum is very thin with a diameter of 0.01-0.02µ. The arrangements of the flagella may be polar or lateral.

Based on the flagellar arrangement, the bacteria are classified as

- Monotrichous bacteria: flagella are present at one pole of the bacteria
- lophotrichous bacteria: a cluster of flagella are present at one pole
- Amphitrichous bacteria : a cluster of flagella are present at both the poles
- Peritrichous bacteria : The flagella are present throughout the surface.

The flagellum contains three parts which are basal, hook, and filament. The basal part is present within the cell wall which contains L-ring (made of lipopolysaccharide), P ring (made of peptidoglycan), S-ring (made of periplasmic space) and M-ring (made of cytoplasmic membrane). The enzymes present in the cytoplasmic membrane provide energy to the flagella for locomotion. The filament is made up of a protein called flagellin. The pili or fimbriae are small hairy like extensions present all over the bacterial body surface. They are used for the attachment to the substratum or other surfaces. The pili may also result in the formation of a conjugation tube which transfers the genes to other bacteria. The bacteria form a gelatinous, amorphous, mesh like capsule. More than one bacteria present in capsule is termed as slime. The capsule provides protection to the bacteria, block the viruses to infect the bacteria, for attachment. The other external structures of bacteria are sheath, prostheca and stalk. The sheath is a hollow tube in which a chain of bacteria exists during unfavourable conditions. Prosthecae are the semi rigid extensions from the rod shaped bacteria which appear during unfavourable conditions. Stalk is the nonliving ribbon like tubular appendages secreted by the bacterial cell.

The internal structure of the bacteria includes cell wall, cytoplasmic membrane and mesosomes. The bacteria consists of a rigid cell wall and it constitutes about 10-40% of the bacterial dry weight. The main constituent of cell wall is peptidoglycan. The peptidoglycan is also called as murin and it is an insoluble porous cross-linked polymer of N-acetylglucosamine, N-acetylmuramic acid and amino acids like L-alanine, D-alanine and D-glutamide. Based on the composition of cell wall the bacteria are classified as gram positive and gram negative bacteria. In gram positive bacteria thick and single layer of peptidoglycan is present along with fewer lipids and other constituents such as are polysaccharides, teichoic acid, and magnesium. In gram negative bacteria the cell wall is very thin, rich in lipids and comprise of two layers, outer lipid membrane and inner peptidoglycan layer. A periplasmic space is present between these two layers. The cell wall constitutes Braun's lipoproteins which join the layers to one another. The outer membrane of the cell wall is made of phospholipid bilayer and lipopolysaccharides. The cytoplasmic membrane is a phospholipid bilayer. The phospholipids constitute about 20-30% of the cytoplasmic membrane and proteins about 60-70%. The cytoplasmic membrane carry several metabolic enzymes and release various extracellular enzymes. Mesosomes are the inward invaginations of the cytoplasmic membrane which are involved in the cell division. The cytoplasm of the bacteria has water, granular ribosomes and chromatin area containing chromosomes, and plasmids. Mitochondria and golgi complex are absent in the cytoplasm. The cytoplasm also consists of other constituents like volutin granules, glycogen, vacuoles etc.,

The bacteria during unfavourable conditions transform themselves into spores. These spore are two types–1. endospores and 2.Exospores. The endospores are formed within the bacteria i.e.,the cytoplasmic area is formed as a spore within the cell wall. The endospores are made of calcium dipicolinic acid. The process of formation of endospores is called sporulation. When the conditions become favourable these spores germinate back to the vegetative form. The exospores are the spore formations outside the bacteria by budding process. Example of the exospores are methylosinus species. Some bacteria like Actinomycetes will form conidiospores and sporangiospores. Azotobacter may form tough and dry cysts during unfavourable conditions.

Study of Fungi

Introduction: Division thallophyta consists of two subdivisions, autotrophic thallophytes e.g. algae and the heterotrophic thallophytes e.g. fungi. The thallus of fungi is known as mycelium and each branch is known as hyphae, with unicellular fungi e.g. yeast (filamentous - thread). Fungi grow mostly in moist conditions and they do not have chloroplast, plastids.

Habitat: mostly saprophytes (fungi grow on dead and decay material) e.g. fungi on bread moulds, parasites, and some are symbionts e.g. lichens, and mycorrhizal association in roots of tracheophytes (from pteridophytes to angiosperms)

Classification: according to Smith fungi are classified into two divisions myxomycophyta and eumycophyta. Myxomycophyta is further classified into three classes myxomycetes (slime moulds), plasmodiophora, and acriceae. Eumycophyta is further classified into phycomycetes (algae-fungi), ascomycetes (sac-fungi), basidiomycetes (club-fungi), and deuteromycetes (imperfect -fungi). The cell wall of fungi is made up of chitin or cellulose, and are aseptate, and coenocytic. The food materials are mostly stored in the form of oil droplets or glycogen droplets (animal starch) in ascomycetes and basidiomycetes. The mycelium is septate, the primary mycelium is monokaryotic and secondary mycelium is dikaryotic.

Digestion: the mode of digestion is in Unit – 13 tracellular or extracellular, in extracellular distribution, the fungi develops haustoria into the host cell, and absorb the food material from the host cell.

Reproduction:

Asexual reproduction: it occurs by

- Fragmentation is the type of reproduction where each fungal hypha cuts into different fragments, which later develop into a new fungi under favourable conditions
- Budding is observed in the yeast where each cell produces small daughter cells known as pseudomycelium

The types of asexual spores are sporangiospores, odio spores, conidiospores, uredospores, teliospores, and ascospores.

Sexual reproduction:

- Phycomycetes, the process of reproduction is anisogamy with isogamy gametangial copulation, where the male gametangia contacts female gametangia through fertilization tube or conjugation tube resulting in the formation of zygosporangium, zygospore, oogospore, oogorium and antheridium e.g. mucor and rhizopus.

- Ascomycete, the female reproductive organ is ascogonium and antheridium, the reproduction do not occur by the fusion of male and female nuclei, instead cytoplasm fusion occurs, with the presence of plasmogamy and absence of karyogamy. Ascospores are produced by the process of meiosis. Both primary and secondary mycelium are present, where the primary mycelium is septate with monokaryotic mycelium and secondary mycelium is aseptate with dikaryotic mycelium which are responsible for the formation of fruit body known as ascocarp.

- Basidiomycetes, the type of reproduction is somatogamy, where the fusion occurs between the vegetative cells, with the absence of gametangial formation where vegetative cells are formed. It undergoes mitosis and forms mitospores, and meiosis to form mesopores. The secondary dikaryotic mycelium is responsible for the formation of fruit body known as basidiocarp.

- Deuteromycetes, is a group of fungi where the sexual reproduction stage is not identified or reported. In the recent research it was identified and categorized these fungi as phycomycetes, ascomycetes, and basidiomycetes.

Study of Virus

A virus is a small non-cellular living particle composed of a nucleic acid (DNA or RNA), which replicates inside a living host.These are obligate parasites i.e. they require a living host for survival. Without the living host the virus may be considered as non living. The morphological features of virus includes, the small size of the virus usually about some nanometres. For example, Poliovirus - 30 nm, Adenovirus - 90 nm, T_4 virus - 225 nm and Ebola virus - 970 nm. The shape of virus may be icosahedral(polyhedron with 20 sides) or helical or complex shapes like cuboidal, spherical and other irregular shapes. The structure of virus is simple. The virus is made up of a protein coat called capsid and is composed of capsomeric units or protein units. The capsid encapsulates the nucleic acid (DNA or RNA) which is the main component of the virus. Some viruses may contain an envelope which is present above the capsid and protects the virus from external environment. Such viruses are called enveloped viruses. Some virus may also contain hairy like projections called spikes which helps the virus to enter and attach the host cell.

Classification

Baltimore classification of virus is widely accepted which classifies viruses into six groups – dsDNA virus, ssDNA virus, ds RNA virus, ss RNA virus with positive strands, ss RNA virus with negative strands, ss RNA virus with reverse transcriptase.

Cultivation of virus

A virus requires a living host for its survival. It replicates and grows in the living host. The living host may be a fertile egg, a living animal, or a tissue culture(animal or plant or bacterial cultures). Now-a-days tissue culture technique is widely used for cultivation of virus. The tissue is grown in a particular media and virus is inoculated into the tissue and during incubation the virus is multiplied. The tissue containing the virus is separated by filtration.

Life cycle of virus

The life cycle of virus consists of two main cycles , lytic cycle and lysogenic cycle. In the lytic cycle the first step is the attachment or adsorption which involves the attachment of the virus to the surface of the host cell. Next step is the introduction of DNA or RNA into the host cell. This leads to the halting of the host metabolism. Then the virus structure get multiplied within the host cell and get assembled. Finally the replicated or the new virus lyse the host cell and come out of the host. The lysogenic cycle includes the attachment and introduction of genetic material into the host cell. Then the genetic material is incorporated within the host chromosome. In this process the viral genetic material does not affect the host cell i.e. the host cell behaves as a normal cell and replicates into a new viral genome and other viral structures. Finally the assembled new viruses undergo lysis and comes out of the host cell.

Study of Rickettsiae

Rickettsiae are the gram negative bacteria which are obligatory parasites i.e., they divide and survive in a living host like ticks, lice, fleas, mites, chiggers and mammals etc.,These are pathogenic in nature. There are four genera of this bacterium – Rickettsia, Ehrlichia, Orientia, and Coxiella and they belong to the order Rickettsiales. The genera Rickettsia are usually non-motile, non spore forming and pleomorphic (change the morphology based on environment) organism. The cocci form has a diameter of 0.1µ and sometimes occur in rod shaped having 1-4µ in length. The survival of the organism depends on the capacity to enter into the host. The diseases caused by this bacteria are typhus fever, rickettsial pox, african bite fever and rocky mountain fever. The genera Ehrlichia belongs to the family Anaplasmataceae and some species are Ehrlichia canis, Ehrlichia ewingii etc. The disease caused by this genus is called Ehrlichiosis which is infected by ticks i.e. zoonotic infection. It is an intracellular parasite and can transfer from cell to cell by filopodia (extensions between the two cells) or by cell rupture. The genera Orientia belongs to the family Rickettsiaceae and the only species present in this genus is Orientia tsutsugamushi. It causes a disease called scrub typhus in humans which is transmitted by the bites or faecal infections. The genera Coxiella belongs to the family Coxiellaceae. The only species present in the genus is Coxiella burnetti which causes Q-fever.

Study of Spirochetes

Spirochetes are a type of gram negative bacteria with lengths between 3 - 500 µm and diameters around 0.09 to 3 µm. These Spirochetes are flexible and are of either spiral or helical curve shaped.Spirochetes are heterotrophic organisms i.e. they do not prepare their own food material and depend on other organic matter for their survival. These are motile but without flagella. The motility is due to the presence of axial fibres or internal pseudo flagella within the cell wall. Spirochetes are very minute and are invisible even in normal microscope. Dark film microscopy and phase contrast microscopy are used to observe spirochetes. The cell wall is very thin and two layered – the outer membrane and the inner P-layer (peptidoglycan layer). In between these two layers a periplasmic area is present in which axial fibres or internal pseudo flagella are present. These reproduce through asexual transverse binary fission. Spirochetes are free living , anaerobic organism and pathogenic in nature. The taxonomical classification for spirochetes is they belong to the order Spirochaetales and families Brachyspiraceae, Leptospiraceae and Spirochaetaceae. The pathogenic diseases caused by spirochetes are Leptospirosis (caused by leptospira species), Lyme disease and relapse fever (caused by borrelia)and Syphilis (caused by treponema species).

Nutritional Requirements

Media is used for the growth of microorganisms which contains all the nutritional requirements. Nutritional requirements for bacteria are

- Energy - chemotrophs (derives energy from chemical substances) and phototrophs (derives energy from radiation, light)
- Electrons - lithotrophs (takes electrons from the inorganic substances) and organotrophs (takes electrons from organic substances)
- Carbon dioxide - autotrophs (utilizes carbon dioxide on its own) and heterotrophic (utilize carbon dioxide from various organic substances)
- Nitrogen - inorganic (utilizes nitrogen from the nitrogen gas) and organic (utilizes nitrogen from organic substances like amino acids and proteins)
- Oxygen - aerobic (utilizes oxygen from the environment) and anaerobic (do not require oxygen for their growth)
- Sulphur and phosphorus are taken from amino acids and nucleic acids
- Metal ions like potassium, calcium, cobalt, manganese, vitamins, growth factors and water are also required.

Various components of medium include -

- Nutrients - carbohydrates, yeast extract, and acidic fluids
- Solidifying agent - agar, the absence of agar is termed as simple broth medium
- Water - distilled water, and demineralised water
- Salt - sodium chloride maintains the isotonicity of medium
- Additives and supplements - these are used only under special conditions

Bacterial Growth

Bacterial growth is determined by the bacterial growth curve, plotting time on x-axis versus bacterial count on y-axis and is given in four phases -

- Lag phase - the bacteria gets adjusted to the media
- Log phase or exponential phase - the division of bacteria takes place increasing the bacterial number exponentially in a short period of time
- Stationary phase - the number of bacteria divided is equal to the number of bacterial cells destroyed
- Decline phase - due to the accumulation of toxins and change in the pH, declination of bacterial cells takes place.

In synchronous growth all the bacteria remain at same stage in their growth cycle, it is imposed in the laboratory by changing its temperature conditions and by membrane filtration technique bacterial culture is filtered with known porosity and all the young bacterial cells are inoculated and cultivated.

Cultivation of Bacteria

Various steps are involved in cultivation of bacteria - preparation of required media, inoculation of bacteria and incubation at required temperature and oxygen. The cultivation is of two types -

- Continuous growth done by supplementing known amount of media continuously into the container and the same quantity is withdrawn and is monitored by two parameters turbid static control and chemostatic control

- Batch growth

Aerobic culturing is mainly used to provide oxygen to the media, liquid media in a medium sized container is placed on a gyratory shaker for mixing, so that atmospheric oxygen present in the container gets dissolved. For large containers spargers are used for the pumping of oxygen into the media continuously, a solid media provides more surface area for aeration. For anaerobic culturing of solid media deep agar tubes are inoculated with bacteria by inoculating needles. Liquid medium contains more amount of oxygen which is removed by viscous media and is subjected for sonication, boiling, addition of cooked meat etc.

Growth and Cultivation of Virus

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Types of Media

The various types of media used for cultivation of bacteria are based on physical nature and are classified into solid agar, liquid and semisolid media containing less than 1 percent.

- Based on cultivation of microorganisms - bacteriological media, and actinomycetes media
- Based on aeration - aerobic and anaerobic media
- Based on chemical substances - simple, basal, complex, synthetic made up of pure chemicals, and natural media (natural substances like egg)
- Based on function - enrichment media extra components like blood agar and selenite

Preparation of medium for cultivation of bacteria involves - accurate weighing of the ingredients and dissolving them in small quantity of water, agar must be heated and filtered and sterilized in autoclave at 121^0C for 15 to 20 minutes, with the adjustment of pH. If required, additives like antibiotics and glucose are added after sterilization. In fungal media the required pH was 5.5 to 6, dextrose at high concentration is used, temperature has to be maintained at 28-30^0C e.g. potato dextrose agar media.

Maintenance of Lab Cultures

The various methods used in the preservation of lab cultures are periodic transfer where a fresh media was prepared and transferred for cultivation. Overlaying of mineral oil protects from evaporation, stays for longer period of time and is reusable. The prepared culture tubes are placed in liquid nitrogen (-195 degrees centigrade) at very low temperature, which results in formation of crystals destroying the organisms, which can be avoided by the use of glycerine and DMSO.

Lyophilization or freeze drying in cultures is done at low temperatures until it is freezed, later subjected to vacuum by slightly increasing the temperature, wherein sublimation occurs with loss of maximum amount of moisture. Desiccation is done to preserve the powder for about 30 years. Soil stocks or inert substances are mixed with sterilized soil with less genetical stability. In harsh conditions bacteria undergoes sporulation, the spores are taken and preserved for longer time.

Isolation of Bacteria

Isolation refers to the separation of a single pure strain from mixed population of microbes such as water, soil, milk etc. Before isolation, selection method is employed to reduce the population of microbes from the sample. The three selection methods are physical method (heat, pH, motility), chemical method (by using inhibitory chemicals), and biological method.

Isolation techniques include - Streak plate method, pour plate method, spread plate method, and micromanipulation method.

- Streak plate method: In this the mixed bacterial culture is inoculated and streaked in unidirectional horizontal and vertical lines across the surface of the agar media, and after incubation one can observe the individual colonies of the bacterium i.e. pure colony.

- Micro manipulation method: In this the sample containing the mixed bacterial culture is placed on the slide and observed under the microscope. The minute micro probes are inserted to the sample on the slide and the required bacteria are picked up, which is then transferred into the fresh medium and cultured.

Identification of Bacteria - Biochemical Methods

Starch hydrolysis test: Starch is a macromolecule found in the bacterial media, some bacteria (Bacillus subtilis) produce enzyme amylase extracellularly which hydrolyses the starch into simpler compounds (dextrin, maltose etc). The bacteria hydrolyzing starch shows positive for the starch hydrolysis test, wherein starch agar media was taken and streaked with the bacterial sample and incubated. After incubation, iodine solution was added onto the agar plate, wherein a colourless zone was observed with the utilization of starch hydrolyzed by the bacteria.

Lipid hydrolysis test: Bacteria like Bacillus subtilis and Staphylococcus aureus produce enzyme lipase which utilizes lipids and hydrolysis them into free fatty acids. In this test, turbid tributyrin agar medium was taken and streaked with the organism and incubated. After incubation, a clear transparent zone was observed with the utilization of lipid by the bacteria.

Casein hydrolysis test: Casein is a milk protein hydrolyzed by the enzyme protease into simple amino acids. Bacteria was streaked onto a milk agar medium plate. After incubation a clear transparent zone was observed with the utilization of lipids by the bacteria and the rest of the agar plates remains turbid. Bacillus subtilis and Pseudomonas show positive casein hydrolysis test whereas E.coli and Enterococcus show negative.

Gelatin liquefaction test: In the presence of enzyme gelatinase, some bacteria hydrolyzes gelatin into simple amino acids. Gelatin at < 20°C appears solid and at >20°C appears liquid. In this test, the nutrient gelatin deep tubes were taken and organism was inoculated. After incubation the temperature of the tubes is reduced to < 20°C, solidified media indicates that the bacteria are unable to utilize the gelatin, and liquid media indicates absence of gelatin with the bacteria utilizing gelatin. Bacillus subtilis, Proteus, and Staphylococcus aureus show positive test and E.coli shows negative.

Indole test: Peptone broth containing tryptophan was used for this test with the bacterial sample being inoculated in the medium. The bacteria that are capable of producing tryptophanase enzyme hydrolyzes tryptophan into indole. The presence of Indole in the broth can be determined by the addition of Kovac's reagent. The appearance of red color indicates a positive indole test i.e. utilization of tryptophan by the bacteria and the appearance of yellow colour indicates a negative test. E.coli shows positive indole test and Bacillus subtilis shows negative indole test.

Methyl red test: Some bacteria has the capacity to accumulate the stable acids after fermentation. MR broth containing glucose was taken and bacteria are inoculated. Upon fermentation, glucose is converted into pyruvic acid and further to other acids like acetic acid with pH below 4.2. With the addition of methyl red indicator, the media turns into red colour indicating the accumulation of stable acids by the bacteria. If the colour resists yellow colour, it is a negative MR test. E.coli and Proteus show positive test whereas Serratia and Enterobacter show negative test.

Voges proskauer test: Some of the bacteria accumulates acetoin, which upon addition of KOH converts into butanediol. MR broth containing glucose was taken and bacteria are inoculated. The glucose present was converted into pyruvate producing acetoin, which upon addition of KOH gives butanediol. The addition of alpha-naphthol gives pink colour depicting that the bacteria is able to convert glucose into acetoin. Klebsiella and Enterobacter show positive test whereas E.coli and Salmonella show negative test.

Citrate utilization test: In this test simmon's citrate media (citrate + indicator) is used which is green in colour, wherein the organism was inoculated and incubated. The bacteria utilizes citrate and hydrolyzes to oxaloacetate and pyruvic acid, with the release of carbon dioxide. The carbon dioxide reacts with the other components of the media forming alkaline compounds. The fluctuation in pH is due to the colour change in the media (green to blue), indicating a positive test. E.g. Klebsiella show positive test whereas E.coli show negative test.

H_2S production test: Some bacteria utilizes sodium thiosulphate and releases H_2S gas, and when H_2S reacts with ferrous sulphate it gives ferrous sulfate which is black in colour with foul smell indicating a positive test for H_2S production test.

Triple sugar iron test: In this triple sugar iron agar medium containing sodium thio sulphate, ferrous sulphate, sugars (lactose, sucrose, glucose) and phenol red indicator were used. The slants of the media were prepared and organism was inoculated. The organisms utilize or ferment the sugars and produce acids which was observed by a colour change (red to yellow), representing positive test.

Catalase test: Some bacteria produce catalase enzyme which converts hydrogen peroxide into water with the evolution of oxygen gas. In this test, bacteria were cultivated in a suitable media. After incubation the organism was placed on the slide with the addition of few drops of hydrogen peroxide. The evolution of oxygen gas indicates that the organism is capable of producing catalase enzyme. E.g. Staphylococcus aureus show positive test.

Urease test: Some bacteria produce urease enzyme that hydrolyzes urea present in the media with an indicator forming ammonium compounds like ammonium carbonate. The pH becomes alkaline with a change in color.

Identification of Bacteria - Staining Techniques

Staining is the process in which a chemical substance, usually an organic compound imparts colour to the micro organisms. As the microorganisms are very minute and colourless, staining techniques are used to identify them morphologically. A stain contains a chromogen and an auxochrome, chromogen consists of an colourless organic solvent and a chemical group chromophore imparting colour, and the auxochrome is a chemical group that conveys the property of ionization. The stain imparts the color by binding with the cellular components like DNA, RNA and other charged molecules.

Types of stains:

- Acidic stains have negative charges, the bacterial cells are negatively charged and they repel the stain, e.g. nigrosin and picric acid.
- Basic stains have positive charge, and the bacterial cells attracts the stain, e.g. crystal violet and methylene blue.

Staining techniques: they are two types - simple staining and differential staining

Simple staining - it is of two types positive staining and negative staining

- Positive staining - basic stains are used where the bacterial cells takes up the stain
- Negative staining - acidic stains are used where the charge is negative with the occurrence of repulsion, wherein the bacteria does not take up the stain and appears colourless

Simple staining is used to study the morphological characteristics of micro organisms.

Differential staining - involves techniques with more than one stain being used. It is used for grouping the bacteria with gram's staining (for gram positive and gram negative bacteria) and acid fast staining (for mycobacterium). For the visualization of special characters flagellar staining, capsule staining, spore staining and nucleic acid staining were used.

Methods:

Positive staining: A bacterial smear was prepared on a glass slide with the help of an aseptic inoculation loop which was fixed with the smear. To this one or two drops of stain was added with the application of heat fixed smear. The excess of smear was removed by washing with water. The slide was then observed under the microscope. As the stain is a basic stain, the bacteria takes up the stain and coloured structures can be observed.

Negative staining: In negative staining to the bacterial smear prepared on a glass slide one or two drops of acid stain was added, and mixed well. Then with the help of a second slide the surface of the first slide was dragged to form a thin film, and dried with no heat fixing and washing being required. The slide was observed under the microscope. As the stain is an acidic stain, the bacteria repels the stain and colourless structures are observed over the coloured dark background.

Gram's staining: It is a type of differential staining discovered by Christian Gram. It is used for grouping of bacteria into gram positive and negative, with the use of four reagents. A bacterial smear was prepared on a glass slide with the help of an aseptic inoculation loop with heat fixing the smear. Crystal violet was added where the bacteria takes up the stain, with the addition of Gram's iodine the primary stain is intensified forming crystal violet-iodine complexes, to which decolourising agent alcohol was added where stage differentiation occurs. Gram negative bacteria has thick lipid layer and upon adding the decolourising agent the lipids get dissolved in alcohol with washing of the crystal violet stain. By the addition of a counter stain safranin the gram negative bacteria appears red in colour. Gram positive bacteria has thick cell wall with the absence of lipids. Therefore the crystal violet colour persists and appears blue in colour even after the addition of counter stain safranin.

Acid fast staining: It is a type of differential staining used for grouping the bacteria into acid fast positive and acid fast negative. It is also called as Ziehl Neelsen staining and is used in the identification of mycobacterium that causes tuberculosis and leprosy. The cell wall of mycobacterium contains rich mycolic acids which doesn't lose the stain once taken. Firstly the smear was prepared to which primary stain carbol fuchsin was added and allowed for steaming. Then the smear was decolourized with acid-alcohol, which differentiates the normal bacterium from mycobacterium. The acid fast bacteria even after treating with acid alcohol retains the carbol fuchsin stain and appears red in colour, whereas the normal cells readily loses the carbol fuchsin stain, upon addition of counter stain methylene blue, cells appear in blue in colour.

Flagellar staining: Flagella is a long appendage in bacteria used for locomotion. In this staining process the glass slide was washed with dichromate, alcohol, isopropyl alcohol and dried, with no heat fixing. A bacterial smear was prepared and air dried, the smear was treated with primary stain, Leifson's stain for about 10 minutes. The stain consists of a dye called Rosaniline and tannin, an astringent, which precipitates the flagellar protein called flagellin. Later, the slide was washed gently and with the addition counterstain Borax-Methylene blue, the cells appear blue in colour with red coloured flagella.

Spore staining: It is also called as Schaeffer Fulton method, where bacterial smear was prepared, primary stain Malachite green was added and subjected to steaming for about 5 minutes. Then this was washed with water, which decolourised the normal cells wherein the spores persists the Malachite green stain. Now, counter stain basic fuchsin was added, the cells appear red in colour with green colour spores. This staining is used to identify the clostridium, anthrax etc.

Bacterial Count -Total & Viable Techniques

Bacterial count can be done by viable count and total count, the viable count is the counting of viable number of bacteria in the given sample which can be done by pour plate method, spread plate method, membrane filtration, and MPN (most probable number) method or multiple tube fermentation method, and in total count, both the viable cells as well as the dead cells are counted.

Different methods used to count the total cells are - direct microscopic method, turbidity method, electron particle counter method, dry weight method, and flow cytometry method.

Pour plate method: In this method, 1 ml of original sample was transferred to 9 ml of sterile normal saline solution, five to six serial dilutions of sterile normal saline solutions were prepared. Now 0.1 ml of each serial dilution sample was transferred into 15 ml of liquid agar test tubes. Liquid agar was transferred into sterile plates before solidification and are incubated. The increase in number of dilutions, decrease the number of colonies. With the consideration of the dilution factor the number of colonies present in the given sample can be given as colony forming units per ml (cfu/ml).

Spread plate method: In this method, 1 ml of original sample was transferred to 9 ml of sterile normal saline solution, five to six serial dilutions of sterile normal saline solutions were prepared. Now 0.1 ml of each serial dilution sample was transferred onto surface of the plates containing agar media, and by using L- shaped spreader the sample was spread and incubated. The first dilution set contains more number of colonies with lesser colonies in the last set. With the consideration of the dilution factor the number of colonies present in the given sample can be given as colony forming units per ml (cfu/ml).

Membrane filtration method: In this method, the sample was thickly populated with the requirement of serial dilutions, and the samples are directly subjected to membrane filtration by taking suitable membrane filters with known porosity. Now, the membrane filters were transferred onto the sterile agar plates. After incubation, the number of colonies formed were counted given as colony forming units per ml (cfu/ml).

MPN method or multiple tube fermentation method: In this method, 1 ml of original sample was transferred into 9 ml of sterile normal saline solution with five or six serial dilutions of sterile normal saline solutions. Now, 0.1 ml of each serial dilution sample was transferred to three tubes containing liquid media i.e. 0.1 ml of first dilution in three tubes and 0.1 ml of second dilution in another three tubes. The results were calculated from each tube with the use of MPN table. Some bacteria like E.coli are able to produce gas by the fermentation of lactose from the media. The accumulated gas was obtained by placing the Durham tubes into the media tubes. The number of Durham tubes containing the accumulated gas can be recorded and the number of E.coli bacteria present in the sample can be calculated from the MPN table.

Direct microscopic method: This method was used to count the total cells present in the sample, a specialized slide called Neuber's chamber was used which is able to fit 0.1 cu mm of sample in each chamber. A coverslip was placed on the chamber which was later observed under the microscope. The number of cells present in each chamber were counted and the total number of bacteria present were calculated from the sample which is given as cells/ml.

Turbidity method: The bacterial sample was taken in the form of a turbid suspension. When a beam of light is passed through the suspension, the light gets absorbed. With an increase in the bacterial count, the turbidity increases with increase in the absorption. This absorption parameter indicates the total number of bacteria present in the suspension.

Electron particle counter or coulter counters method: In this, a device having a small orifice separates the positive and negative electrodes. When the bacterial sample is passed through the orifice, a signal passes between the two electrodes which counts the total number of electrodes present in the sample.

Dry weight method: In this method, the sample solution is filtered across the membrane filter. The weight of the membrane filter was measured before and after the filtration. The difference between the weights of the filter gives the dry weight of the cells which will be the total number of bacterial cells present in the sample.

Flow cytometry method: In this method, the flow of the bacterial sample is detected by using laser beam, which gives both the total number of bacterial cells present in the sample and the 3D image of the cells.

Methods of Sterilization

Sterilization is a process of eradicating all forms of live microorganisms in a substance. An antiseptic destroys any type of infection living on the tissue, and disinfectants are meant to destroy microorganisms that infect non-living objects. Sterility assurance is level 10^{-6} (1 in a million). Sterilization can be classified as destruction, denaturation, inactivation and removal of microbes. Sterilization can be physical (heat- dry or moist autoclaving and by radiation- ionizing e.g. X-rays, γ-rays etc. and non-ionizing e.g. UV radiations), chemical (gas or fluid) and mechanical (air or liquid filtration using cellulose membrane filters, asbestos Seitz filters and diatomaceous candle filters). Surgical instruments -boiling, incineration and autoclave, glass ware- autoclave, boiling and hot-air oven, water- filtration, powders and dry forms- it is hot air oven if thermo stable if not gaseous methods and radiation, hospitals are for surgical metallic instruments boiling- boiling, autoclave, incineration.

Factors affecting sterilization - number and type of microbes, temperature and pH and presence of organic matter (coating), time and concentration have linear effect of sterilization and mode of action. Relative resistance of microbes to agents of sterilization - highly resistant (spores of bacteria, prions), moderate resistant (protozoa cysts, Staphylococcus aureus, Mycobacterium tuberculosis, and Pseudomonas) and least resistant (Protozoa, enveloped virus, fungal spores and vegetative bacteria).

Dry heat sterilization mechanism is by protein denaturation, oxidative damage and toxic effect of elevated electrolyte (in absence of water). The conditions of dry heat sterilization are 160^0C for 1 to 3 h, 170^0C for 1 to 2 h, and 180^0C for 0.75 to 1 h. Moist heat autoclaving (T0 - time - pressure = 100^0C for 90 min at 0 LBs, 110^0C for 60 min at 5 LBs, 116^0C for 30 to 40 min at 10 LBs, 121^0C for 15 to 20 min at 15 LBs, 126^0C for 10 min at 20 LBs and 135^0C for 3 min at 30 LBs), acts by denaturation and coagulation of proteins and has pre-vacuum sterilization and post-vacuum periods. Radiation (cold sterilization) causes damage to the DNA of the microbe. Gaseous sterilization (using ethylene oxide, hydrogen peroxide and formaldehyde) causes alkylation.

Sterilization Methods for Pharmaceutical Products

Sterile products are the products subjected to sterilization with no micro organisms in it. Examples include parenterals, dressings, sutures, materials or instruments used during surgery, ophthalmic products, irrigation fluids used for irrigating tissues, dusting powders, ointments and creams, dialysis solutions, implants, culture media, filters, air, fermentation vessel and media and equipments. All these are categorized under two categories. Products that are sterilized in the final container is called terminal sterilization (autoclave and gamma radiation sterilization) and the other is aseptic processing, where sterilization is required separately for the product, closure, container and other articles.

The commonly used sterilizations are dry heat and moist heat, radiation and filtration sterilization, and others like UV. The moist heat comes under terminal sterilization, and filtration comes under aseptic processing. The moist heat sterilization is not suitable for non aqueous preparations, and dry heat is used for products that can withstand high temperatures (glass articles).

- Ethylene oxide sterilization is done for heat labile products which cannot withstand heat sterilization and filtration is used for bulk liquids and gases
- Injections or parenterals, aqueous solutions in small quantity which can withstand high temperature can be filled into vials or ampoules, which are then subjected to terminal sterilization by autoclaving
- Oily injections, small volume are subjected to dry heat sterilization
- Suspensions (both aqueous and oily suspensions) are subjected to terminal sterilization
- IV fluids of high volume can be sterilized by using autoclave or by terminal sterilization or by filtering
- TPN (total protein nutrition) by aseptic processing
- Closures are sterilized by radiation sterilization
- BFS (blow filled sealed containers) used for vaccines, anaesthetics etc which involves multi step processes are sealed aseptically
- Non injectables like irrigation fluids, dialysis fluids are needed to be sterilized by an autoclave
- Ophthalmic preparations are sterilized by aseptic processes
- Dressings containing cellulose are sterilized by autoclaving, if it withstands the temperature
- Wax containing materials has to be sterilized by dry heat sterilization
- Implants present in small cylinders, which contain some hormones and polymers withstanding the temperature are sterilized by autoclaving and dry heat sterilization
- Sutures and ligatures, in case of absorbable sutures, are sterilized by terminal sterilization using gamma radiation
- Non absorbable sutures are sterilized by autoclaving

Sterility Testing of Pharmaceutical Preparations

Sterility testing of pharmaceutical preparations involves a test to assure whether the pharmaceutical preparation is free from contaminating microorganisms or not. The sterility testing is usually done for the viable organisms.

Limitations of sterility testing: The process is a statistical process i.e. only few samples are tested for sterility and are assumed for whole batch. If the sterility testing was carried out in a medium which do not support the growth of the organism, then the product is sterile. If the organism number is less, the chance of detection is also low and the product is considered to be sterile.

Methods of sterility testing: It can be done by two methods, direct method and indirect method, the direct method is the method where the product is directly inoculated into the medium and incubated, the growth of the organism is observed. Indirect method is the membrane filtration method. Apart from these two methods another test is known as sensitive method which is used to detect low level of contamination in large volumes like intravenous fluids.

Requirements for sterility testing: The requirements for sterility testing are - medium, supports the growth of organism (e.g. fluid thioglycollate medium supports the growth of anaerobic bacteria, and soya bean digest casein medium supports the growth of aerobic bacteria), positive control (with organism) and negative control (without organism), with the sampling being done according to the pharmacopeia.

Direct inoculation method: In this method, the product is soluble in media, and is directly inoculated into the medium and incubated for 14 days, with the growth of the organism being estimated. If the product is insoluble in media, then the product is oily or waxy and should be diluted in diluting fluids, e.g. Fluid A (peptic digest of animal tissue in water having pH 7.1) or Fluid B (peptic digest of animal tissue in water and polysorbate 80).

Indirect method or membrane filtration method: The product which has to be tested for sterility is filtered through a hydrophobic membrane filter. Fluid A or Fluid B is used to dilute or solubilize the products, the membrane is cut into two or three pieces and are washed with fluid A, placed in the media, inoculated and observed for the growth of microorganisms. This method is also done for the products with bacteriostatic activity like preservatives, antibiotics etc. This method is advantageous over direct inoculation method as it can determine fewer organisms present in the sample and can be done for large volumes of product.

Other tests used are pyrogen test and LAL (Limulus Amoebocyte Lysate) test for sterility of products.

Observation and interpretation: The positive control must show growth and negative control should show no growth. If the sample does not show any growth then the test is considered to be passed i.e. the product is sterile and if the sample shows growth then a re-test is done for sterility. In the retest if the sample does not show any growth then the test passes, and if the sample shows growth then second re-test is done, and in the second retest if the sample shows growth then the batch is rejected.

Brief Information on Validation

Basic principles of validation are:

I. Installation qualification - to ensure that equipment used for sterilization is installed properly.
II. Operational qualification - calibration and testing of instrument is done to ensure the proper working of the instrument.
III. Performance qualification - to ensure the completion of actual sterilization methods and to check whether the actual conditions are achieved or not. It is normally done by the biological, chemical or physical indicators.

Types of indicators:

Physical indicators: Physical indicators involve observing the display on the sterilizer and to record the time, temperature and pressure.

- Dosimeter is physical indicator used in the radiation type of sterilization. When a dosimeter is kept in a container, a change of color is observed if the proper dose of radiation is present.
- DOP testing: It is used for air filtration in laminar air flow. DOP testing is a process in which the integrity of HEPA (High efficiency particulate air) filter is tested through the introduction of particulates. DOP when burnt forms smoke particles. When air is passed through the HEPA filters, smoke is added to the passage and blockage of pores is checked. If there is 99.9% of blockade, then it indicates the HEPA filters are capable to retain any particles which are above 0.3μ.
- Bubble pressure test: The pressure at which the first bubble appears is the bubble point pressure. It mainly indicates the pore size of the membrane filter and helps in the identification of the sterilization procedure.

$$D = 30_{\gamma}/P$$

D = pore size, $_{\gamma}$ = surface tension and P = pressure

Chemical indicators: These are the substances which gives the visibility by change in color.

- Browne's tube: It is a chemical indicator with a heat sensitive solution within the glass tube which changes from red to green when tube is subjected to high temperature. It is used for monitoring dry heat & sterilization of fluid's process.
- Bowie dick tape is an adhesive tape used in autoclaving (heating under high pressure with steam) to indicate the specific temperature.
- Witness tube in an autoclave contains some crystals, which melts when the final temperature point is achieved. Compounds like sulphur, acetanilide and benzoic acid are used.

Biological indicators: These are used in all types of sterilization process. It is also known as the spore test. Resistance spores with suitable organisms are taken in tubes and are taped. These tubes along with the sterilizing substances are finally sterilized. After sterilization, spores are checked for viability. These are done by taking spores in a medium, and incubating them. If there is no growth of organism, it indicates spores are killed and sterilization is done.

- Geobacillus stearothermophilus is used for heat sterilization. After sterilization, it is kept in a special broth and usually grows at higher temperature of 55 for five days.
- Bacillus subtilis is used for dry heat sterilization.
- For ethylene oxide sterilization or gaseous sterilization, bacillus subtilis bacteria is used.

In filtration type of sterilization, biological indicators are used. Organisms like serratia marcescens and brevundimonas diminuta are very small in size. If the organisms are retained on the membrane, it indicates filtration is done properly.

Ideal Properties of Disinfectants

Ideal Properties of disinfectants include

- It should be non-toxic, and non-irritant
- It should be soluble in water
- It should be cheap and easily available
- It should be active against all organisms, easily spreadable, and should have a quick action in destroying microorganisms
- It should have good fragrance, and good penetration

Types of Disinfectants

The various types of disinfectants used are

- Phenol - causes physical damage to the cytoplasmic membrane and destroys organism, and are active on vegetative forms rather than spores. Phenols are used as disinfectant, and antiseptic mostly in hospitals.
- Heavy metals like silver mercury copper also have antibacterial action which form complex with the sulfite group and destroy microorganisms and act as both bactericidal and bacteriostatic.
- Dyes like crystal violet, acriflavine are used at low concentrations as they form stains.
- Quaternary ammonium compounds like cetrimide, benzalkonium chloride etc. remove dirt and microorganisms, by formation of lather, by denaturing the proteins in the microorganism and by membrane damage.
- Aldehydes like formaldehyde and glutaraldehyde are bactericidal, used in aseptic rooms for storing the specimens in order to avoid the decomposition of organs, and also in medical field for sterilization.

Evaluation of Disinfectants

Evaluation of disinfectants is done by three methods

1. Phenol coefficient method - Reidel walker and Chick-martin test are used where new disinfectant is taken and compared with phenol.

2. Determination of minimum inhibitory concentration (MIC) required for the inhibition, and it is of two types liquid dilution method and solid dilution method.

3. Toxicity test to evaluate the toxicity of disinfectants which includes acute toxicity and chronic toxicity.

Defination, Classification and Types of Immunity

Immunity is the ability or resistance of the host or an organism to fight against the microorganisms and their toxins. The primary function of body's immunity is to defend against the invaded foreign material, eliminate them and to minimize the damage to the body. The immune system of the body depends on the ability to discriminate between foreign cells (non self) and the host cells (self). Years ago, a British scientist, Edward Jenner observed that one who gets cowpox are protected from smallpox. The person will develop resistance so that it does not cause smallpox and this is developed as a vaccine against the disease. After that many scientists have made great progress to develop vaccines against several diseases.

Anything that is foreign to the body is called an antigen. The antigen may or may not be harmful to the body. The disease causing antigen is known as pathogen and the ability of the pathogen to cause the disease is called virulence. The reduction in the virulence of the pathogen is called attenuation leading to an avirulent pathogen. Whenever an antigen enters the body, the immune system produces antibodies (immunogens or immunoglobulins) against the antigens. Antigens have certain determinants called epitopes onto which antibodies can bind. Hapten is a small-molecule and capable to bind to the antibody, but usually doesn't initiate an immune response. Immunoglobulins or antibodies are of two types - Monoclonal immunoglobulins (which react with one epitope of antigen) or polyclonal immunoglobulins (which react with many epitopes).

The immune system is typically divided into two types – Innate immunity (nonspecific) and Adaptive immunity (specific). Innate immunity is further divided as cellular and humoral innate immune systems. The adaptive immunity refers to antigen-specific immune response. The adaptive immunity is again divided as, cell mediated adaptive immune system and humoral mediated adaptive immune system. Innate immunity is naturally present in the body. When a foreign material or antigen or pathogen enters the body, the external or first line of defence mechanism i.e., skin, secretions, mucous membrane will try to prevent the entry of foreign organisms. If the foreign substances able to enter the body breaking the external defence mechanism, the internal or second line of defence mechanism gets activated by producing phagocytes, natural killer cells (NK cells), antimicrobial proteins etc within the body. All these are included in the innate immune system. If the antigen is able to overcome the innate immunity, the adaptive immunity tries to fight against the antigen which includes various types of T-cells, B-cells and memory cells.

Natural Immunity

Natural immunity or innate immunity is the natural defence of the body which protects the body from the invading micro organisms. The innate immune system is the first line of defence against invading organisms. In addition, the innate immune system also has anatomical features that function as barriers to infection. It is not antigen specific and is different in different species, races, and individuals.

The natural immunity gives external defence and internal defence. The external defence mechanism may be due to the mechanical barriers (skin and cilia) and secretions (saliva, lacrimal fluid, and mucous). The internal defence mechanism includes phagocytes, complement system (the activated complement can lead to increased vascular permeability, recognition by phagocytic cells, and lysis of the foreign organism), interferons (proteins that can limit virus replication in cells), natural killer cells (NK cells non-specifically kill virus infected and tumor cells, macrophages, neutrophils, and eosinophils), and interleukin-1 (induces fever to opsonize bacteria).

Adaptive Immunity

Adaptive immunity is also called as acquired immunity that can be acquired during lifetime. It can be classified as Humoral Immunity and Cell mediated immunity. In Humoral immunity; the effector cells are B-cells and immunises the host by production of antibodies which can form complex with antigen (Ag-Ab complex) that filter out foreign substances. In Cell mediated immunity; T-cells are majorly involved and is driven by antigens unlike Humoral immunity. Major Histocompatibility Complex also helps in driving out foreign substances. Hence, Cell mediated immunity is also callled as Non-Antibody mediated immunity. B-cells mature in bone marrow and T-cells mature in thymus cells. Antigen Presenting Cells (APCs) also play a major role in immunity but do not respond to any specific antigenic stimulus. These cells in response to stimulus produce certain chemicals like Cytokines (chemical messengers), Lymphokines (soluble proteins), Interferons, Interleukins.

Humoral immunity refers to the component of the adaptive immune response that is caused by B cells, antibodies, and type 2 helper T cells, as well as circulating mast cells and eosinophils to a lesser extent. Type 2 helper T cells are included in the humoral immune system because they present antigens to immature B-cells, which undergo proliferation to become specific to the presented antigen. The B cells then rapidly produce a large number of antibodies that circulate through the body's plasma. Antibodies provide a number of functions in humoral immunity. There are six different classes of antibodies that provide distinct functions and interact with different cells in the immune system. All antibodies bind to pathogens to opsonize them, which makes it easier for phagocytic cells to bind to and destroy the pathogen.

Cell-mediated immunity is controlled by type 1 helper T cells and cytotoxic T cells. These cells are activated by antigen-presenting cells (APCs), which cause them to rapidly mature into forms specific to the antigen. T cells circulate through the body to destroy pathogens in several ways. Helper T cells facilitate the immune response by guiding cytotoxic T cells to pathogens or pathogen-infected cells, which are destroyed later. Cytotoxic T cells kill pathogens in several ways, including the release of granules that contain the cytotoxins perforin and granzyme, which lyse small pores in the membrane of a pathogen. T-cell produced proteases enter the pathogen and induce an apoptosis response within the cell. Helper T cells secrete cytokines such as interferon-gamma, which activates cytotoxic T cells and macrophages.

Acquired Immunity-Active & Passive

Immunity is obtained either from the development of antibodies in response to exposure to an antigen, as from vaccination or from an attack of an infectious disease, or from the transmission of antibodies, as from mother to fetus through the placenta or through the injection of antiserum. Immunity is not inherited. Acquired immunity can be active or passive, active immunity results from the development of antibodies in response to an antigen, as from exposure to an infectious disease or through vaccination, whereas passive immunity results from the transmission of antibodies, as from mother to fetus through the placenta or by the injection of antiserum.

Acquired immunity is divided into two types natural acquired immunity and artificial acquired immunity.

Naturally acquired active immunity - It occurs when the person is exposed to a live pathogen, develops the disease, and becomes immune as a result of the primary immune response. Once a microbe penetrates the skin, mucous membranes, or other primary defences, it interacts with the immune system. B-cells in the body produce antibodies that help to fight against the invading microbes.

Natural acquired passive immunity - Maternal passive immunity is a type of naturally acquired passive immunity, and also refers to antibody-mediated immunity conveyed to a fetus or infant by its mother. Naturally acquired passive immunity can be provided during pregnancy, and through breast-feeding. In humans, maternal antibodies (MatAb) are passed through the placenta to the fetus by an FcRn receptor on placental cells.

Artificially acquired immunity - the person must be artificially and intentionally exposed to foreign antigens (actively), or is given someone else's antibodies (passively), in order to generate a protective immune response.

Artificially acquired active immunity - is the protection produced by intentional exposure of a person to antigens in a vaccine, so as to produce an active and lasting immune response. The antigens in the vaccine stimulate the immune system to produce antibodies and memory cells which are specifically directed against the antigens in the vaccine. After the immunization, if the living infectious agents with the same antigens that were in the vaccine get into the person's body, the correct antibodies already present binds to the infectious agents.

Artificially acquired passive immunity - is the protection acquired by giving a person an injection or transfusion of antibodies made by someone else. These antibodies neutralise the infectious agents in the usual way, but the protection lasts only a few weeks because the antibodies gradually break down and are not replaced. In artificial passive immunization there is no involvement of the person's own immune system.

Vaccine doses - Some vaccines are given as a single dose, but others as a course of three doses at intervals of a few weeks. Some vaccines also require a 'booster dose' five to ten years after the original immunization. This is necessary to increase the immune response and ensure an adequate level of protection. Once established, the protection provided by immunization usually lasts for several years, or even for life. This makes immunization a highly effective method of giving long-lasting immunity.

Phagocytosis

Phagocytosis is a specific form of endocytosis involving the vascular internalization of solids such as bacteria by an organism, and is therefore distinct from other forms of endocytosis such as the vesicular internalization of various liquids (pinocytosis). Phagocytosis is involved in the acquisition of nutrients for some cells. The process is homologous to eating at the level of single-celled organisms; in multicellular animals, the process has been adapted to eliminate debris and pathogens, as opposed to taking in fuel for cellular processes, except in the case of the animal Trichoplax. In an organism's immune system, phagocytosis is a major mechanism used to remove pathogens and cell debris. For example, when a macrophage ingests a pathogenic microorganism, the pathogen becomes trapped in a phagosome which then fuses with a lysosome to form a phagolysosome. Within the phagolysosome, enzymes and toxic peroxides digest the pathogen. Bacteria, dead tissue cells, and small mineral particles are all examples of objects that may be phagocytosed.

Mechanism - Cells have evolved a whole host of mechanisms for ingesting particles and fluids. These vary from receptor-mediated endocytosis (absorption of small particles into clathrin-coated vesicles), to pinocytosis (the uptake of soluble material), to phagocytosis. Phagocytosis is the mechanism by which relatively large particles, such as bacteria, dead cells, or polystyrene beads are engulfed. During phagocytosis, in immune cells such as neutrophils and macrophages, receptors in the cell membrane first recognize antibodies on the target, which causes membrane protrusions called pseudopodia to surround the target in a zipper like mechanism. This is followed by fusion with lysosomes, acidification of the phagosome, and degradation of the target.

The most widely-studied example of phagocytosis involves Fcγ receptors, which recognize particles coated with immunoglobulin. Fcγ receptors are expressed in white blood cells in four different classes, distinguished by their antibody affinity. Upon binding to the Fc region of IgG, receptors signal via Syk kinases, small GTPases, and hundreds of other molecules, leading to substantial reorganization of the actin cytoskeleton and its contraction by multiple myosin isoforms. The resulting complexity has the potential to obscure the fundamental processes and principles that, as often is the case in biology, may well be quite simple. In particular, the connection between fundamental physical mechanisms and biological regulation remains to be explained. Focusing on only the most important components, such as the receptors, ligand density, and particle shape, may help elucidate the fundamental underlying mechanisms of engulfment.

Chemical Nature of Antigens

Antigen is a substance which stimulates the production of an antibody when introduced into the body. An antigen may be a substance from the environment, such as a chemical, protein, polysaccharide, bacteria, virus, or pollen. Antigen has two properties, immunogenicity (tend to stimulate immune system and produce antibodies) and reactivity (reaction with an antibody). The complete antigens are those that contain the properties of immunogenicity and reactivity, whereas incomplete antigens or haptens are those only with reactivity. Immunologically active regions of an antigen that binds to specific membrane receptors on lymphocytes or to antibodies are called as antigenic determinants or epitopes.

Properties of antigen: The factors influencing the antigenicity are -

- Foreignness - an antigen must be a foreign substance to the body to elicit an immune response.
- Molecular size - active antigens have large molecular size, and depending upon the size, the antigen may be complete or incomplete antigen.
- Chemical nature - antigens are mainly proteins, with some being polysaccharides. The more complex the antigen structure, the more is the immunogenicity.
- Physical nature - particulate antigens are more immunogenic than soluble ones and denatured antigens are more immunogenic.
- Degradability - antigens that are easily phagocytosed are generally more immunogenic because, most antigens that develop an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).
- Genetic factors - some substances are immunogenic in one species but not in another.
- Age - age can also influence immunogenicity.
- Dose - administration of an immunogen can influence its immunogenicity, the lesser the dose the less the immunogenicity.
- Route of administration- the route of antigen administration alters the nature of the response.
- Adjuvant - adjuvants are the substances that are non-immunogenic alone but enhance the immunogenicity of any added immunogen.

Types of antigens:

a) Based on nature - proteins, polysaccharides, nucleic acids, and lipids

b) Based on immune response - complete and incomplete antigen (haptens)

Bacterial Toxins (Exotoxins and Endotoxins) and their Significance

Bacterial toxins are the toxins produced by microorganisms, including bacteria. These toxins promote infection and disease by directly damaging the host cell. The capacity of microorganisms to produce toxins is known as toxigenicity, and the toxin present in the blood is called toxemia. Antitoxin is an antibody which is capable of neutralizing the specific toxin. Toxoid is an inactivated or suppressed bacterial toxin which can be used as a vaccine. The toxins are of two types – exotoxins and endotoxins.

Exotoxin is a toxin formed and excreted by the bacterial cell, and is released into the surrounding medium and is the most poisonous substance. These are mostly protein compounds which causes various diseases, and are mainly produced by gram positive bacteria, sometimes from gram negative organisms. These have a molecular weight of 10 KDa.

- Cytotoxin is a type of exotoxin which kills the host cell by attaching to the cell membrane.
- Neurotoxin interferes with the nerve impulse.
- Enterotoxin is an exotoxin which affects the gastrointestinal tract.

All the exotoxins can be denatured with anti toxins and toxoids can be prepared. These are relatively unstable, heat labile (60°C), highly antigenic, and highly toxic. They usually bind to specific receptors with no enzyme activity, and are filterable. Exotoxins can be detected by neutralization and precipitation tests. Examples: Toxins produced by Staphylococcus aureus, Bacillus cereus, Streptococcus pyogenes, Bacillus anthracis etc and cause diseases like Tetanus, diphtheria, botulism etc.

Endotoxin is a toxin formed inside the bacterial cell and is released only on cell lysis. These are lipoidal in nature i.e. lipopolysaccharides produced by the gram negative bacteria. These have large molecular weight of about 50-1000 KDa. When the bacterial cell is lysed, along with the endotoxins some pyrogens, cytokines are also released which cause fever, shock, blood clotting, and even death. Endotoxins cannot be neutralized with anti toxins and toxoids cannot be prepared. Endotoxins are relatively stable, heat tolerant and weakly immunogenic. These do not have specific receptors and specific enzyme activity and are non filterable. These are detected by Limulus lysate assay. Examples: Toxins produced by E.coli, Salmonella Typhi, Shigella, Vibrio cholera etc and cause diseases like Typhoid, cholera, Meningococcemia, sepsis etc.

Structure and Formation of Antibodies

An antibody or immunoglobulin is a Y-shaped protein produced mainly by plasma cells and B-cells that help in identifying and neutralizing the foreign substances. Antibodies are distinguished into five different classes as IgM, IgD, IgG, IgA, and IgE. The antibody consists of four polypeptide chains. Two heavy chains and two light chains joined to form Y-shape. The chains are attached by the disulphide bonds. The amino acid sequence at the tips of the Y portion is called Variable portion which is the antigen binding site. The rest of the portion is called constant portion which helps in binding to the complement. Heavy chains are of five types they are - α, δ, ε,γ, and μ and have a size of 50 KDa. Light chains are of two types namely, lambda (λ) and kappa (κ) and have a size about 25 KDa. The variable portion has about 100 amino acids. When an antibody is digested with pepsin, it is divided into two portions i.e. Fab (fragment, antigen-binding) and Fc regions. When it is digested with papain, it is divided into three portions, two Fab (fragment, antigen-binding) fragments and one Fc fragment. When digested with mercaptoethanol, the disulphide bonds breaks and it is divided into heavy and light chain portions. The different types of antibodies are based on the type of heavy chain. IgG has 'γ' heavy chain, IgM has 'μ' heavy chain, IgA has 'α' heavy chain, IgD has 'δ' heavy chain, IgE has 'ε' heavy chain.

IgG antibody is a monomer which constitutes 80% of serum antibodies. These are present in blood, lymph, and intestine. They get activated by the complement, enhance the phagocytosis, neutralize the toxins, they can cross the placental barrier and protect the foetus. The half life of IgG antibodies is 23 days. IgM has a pentameric structure and each unit is joined together by a J-chain. These constitute about 5-10% of serum antibodies. These are responsible for agglutination and the half life is small i.e., 5 days. IgA is a dimer and both the units are joined by a J-chain. These constitutes about 10-15% of serum antibodies. These are responsible for secretions and tend to protect the mucosal layer. The half life is about 6 days. IgD and IgE are monomers and constitutes about 0.2% of serum antibodies. The half life is 3 days. These are present in mast cells, basophils, and blood. These initiate allergic reactions.

When a foreign particle enters the human body and if it crosses the first line of defence and second line defence mechanism, then the T-lymphocyte promotes the production of IgD antibodies which will be recognised by the B-cell and IgM antibodies are produced. Then the B-cells are cloned and activate the IgG antibodies followed by the production of some IgA and IgE antibodies.

Steps involved in production of antibodies:

1. Attachment of antigen to macrophage and MHC (which recognises the antigen).

2. Activation of T- helper cells followed by the activation of B-cells in which antibodies are present.

3. The antigen binds to the antibody forming antigen-antibody complex.

4. The B-cells proliferate forming plasma cells which produce more antibodies (produce IgD, IgM, and then IgG).

5. The antibodies enter into the bloodstream and neutralise the antigens. Some B-cells produce memory cells which recognise the antigens when invaded again and produce the antibodies.

Antigen - Antibody Reactions

The specific interaction between the antigen and antibody results in antigen-antibody reaction. This forms the basis for antibody mediated immunity. The antigen-antibody reaction forms a complex, wherein the antibody inactivates the antigen. The inactivation of antigen can be done by neutralization method, agglutination method, precipitation method, and complement fixation. These three methods enhance the phagocytosis and the inflammation finally leads to the destruction of antigen.

Agglutination method: when particular antigens are mixed with its antibodies, they form clumps or agglutination. The agglutination reactions are done qualitatively and quantitatively. Different types of agglutination tests are direct, indirect, flocculation and tube agglutination tests etc.

- Direct agglutination test is also called slide agglutination test, which is used for blood grouping (haemagglutination)
- Tube agglutination test is the standard test for quantitative estimation of antigens. Classical example of tube test is Widal test done for the diagnosis of typhoid.
- Indirect agglutination test is a passive agglutination test used to diagnose rheumatoid arthritis. Coomb's test is a type of flocculation test which is done to determine the Rh factor.

Precipitation method: If the antigen is soluble, it results in the precipitation of the antigen when reacted with a particular antibody forming insoluble antigen-antibody complex. The various types of precipitation tests are

- Radial immunodiffusion test - in this the antibody is incorporated into the agar gel and different dilutions of the antigen are placed in holes punched into the agar. As the antigen diffuses into the gel, it reacts with the antibody and a ring of precipitation is formed.
- Immunoelectrophoresis - in immunoelectrophoresis, a complex mixture of antigen is placed in a well of an agar gel and the antigens are electrophoresed so that the antigens are separated according to their charge. As the antibodies diffuse into the agar, precipitin lines are produced when an antigen-antibody reaction occurs.
- Radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) - in this a radiolabeled or enzyme labelled antigen is linked to specific antibody.
- Immunofluorescence - Immunofluorescence is a technique whereby an antibody labelled with a fluorescent molecule (fluorescein or rhodamine or one of many other fluorescent dyes) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.

Complement fixation: Tests for antigen/antibody complexes based on the consumption of complement are termed complement fixation tests and are used to quantitate antigen/antibody reactions. This test works only with the complement fixing antibodies (IgG and IgM). It is used in diagnosing syphilis. The antigen is mixed with the test serum, with the formation of antigen-antibody complexes. The presence of antigen/antibody complexes fixes the complement and reduces the amount of complement in the tube. After allowing complement fixation by any antigen/antibody complexes, a standard amount of red blood cells, which have been precoated with anti-erythrocyte antibodies, are added. If all the complement was still present i.e. no antigen/antibody complexes formed between the antigen and antibody, all the red cells will be lysed. If antigen/antibody complexes are formed between the antigen and antibody, some of the complement will be consumed and, when the antibody-coated red cells are added not all of them will lyse. By measuring the amount of red cell lysis, and by measuring the release of hemoglobin into the medium, one can indirectly quantitate antigen-antibody complexes in the tube.

Applications of antigen-antibody reaction:

- Determination of blood groups
- Determination of different infective agents
- Development of various immunoassays

Immunization/Vaccination with Importance of Booster Dose

Vaccination or immunization is an administration of live or killed antigenic material in order to stimulate the immune system against the microbe, thereby preventing disease. Vaccine is antigenic but not pathogenic.

Types of vaccine:

1. **Live or attenuated vaccine –** it can be bacterial or viral. Examples-BCG (Bacillus Calmette-Guerin) is a live or attenuated bacterial vaccine used for tuberculosis (TB) disease. Polio vaccine is a live viral vaccine which is two types -Sabin and Salk vaccine. Sabine is the oral attenuated polio vaccine.

2. **Killed or inactivated vaccine –** bacteria or viruses are inactivated with heat and/or chemicals (usually formalin). Examples-Cholera, typhoid vaccines are bacterial killed vaccines. Rabies and Salk polio vaccines are killed viral vaccines.

3. **Toxoid vaccine –** these are the toxins of bacteria which are made harmless to initiate immune response. Examples-Diphtheria and tetanus vaccines.

4. **Subunit vaccine -** vaccines from parts of the cell like polysaccharide, proteins and peptides. Examples - Neisseria meningitidis, Streptococcus pneumoniae are polysaccharide vaccines and Hepatitis vaccine is a protein vaccine.

5. **Recombinant vaccines -** vaccines from surface antigens by using genetic engineering technology. Examples-Hepatitis B vaccine.

6. **DNA vaccines –** influenza, papillomavirus vaccines.

BCG vaccine (Bacillus Calmette-Guerin): It is a live or attenuated bacterial vaccine used for tuberculosis (TB) disease, meningitis. It consists of live attenuated strains of Mycobacterium bovis and subcultured in a specific medium. It is a long term vaccine and is stored in ampoules and refrigerated at 2-8°C. The dosage will be 0.05-0.1 ml.

OPV (Oral Polio Vaccine): It is the oral attenuated polio vaccine developed by Sabin. The vaccine is to be given in multiple doses upto 5 years of age.

IPV (Inactivated polio vaccine): It is a killed polio vaccine developed by J. Stalk in 1955. It requires only two doses and is more thermostable. It is grown in monkey kidney tissue culture and is then inactivated with formalin.

DPT vaccine: It is trivalent vaccine, protects against three infectious diseases in human i.e. Diphtheria, Pertussis, and Tetanus. Diphtheria is caused by Corynebacterium diphtheriae; tetanus is caused by Clostridium tetani, and pertussis is caused by Bordetella pertussis. The vaccine is prepared by taking the toxoids of diphtheria and tetanus and killed whole cells of pertussis bacteria. The toxoids are mixed with aluminium hydroxide and then mixed with the killed bacteria of pertussis. It is given as booster dose upto 5 years and later.

Measles vaccine: Measles can be prevented with the MMR (measles, mumps, and rubella) vaccine. It is an attenuated vaccine. Children get two doses: the first dose at 12 through 15 months of age and second dose between 4-6 years.

Typhoid vaccine (TAB): It may be live or attenuated vaccine prepared from S.typhi, S. paratyphi A and B strains. There are two vaccines to prevent typhoid. One is an inactivated (killed) vaccine administered in injection form, and the other is live, attenuated (weakened) vaccine, which is taken orally.

Hib vaccine: Hib vaccine prevents infections caused by a bacteria called Haemophilus influenzae type b. The vaccine is prepared from capsule polysaccharide of the bacteria.

Varicella vaccine: It is used to prevent chickenpox. It's also called as the varicella vaccine, as chickenpox is caused by the varicella-zoster virus. The vaccine is made from a live but weakened or attenuated virus.

Hepatitis vaccine: It is used to prevent hepatitis. The vaccine may be a subunit vaccine or recombinant vaccine.

Immunization programme: WHO has rapidly expanded and strengthened disease detection and eradication like polio, diphtheria, tetanus, pertussis, tuberculosis, measles, hepatitis, meningitis, cholera, typhoid. The schedule of immunization programme is,

1. BCG (Bacillus Calmette Guerin) 1 dose at Birth, intradermally.

2. OPV (Oral Polio Vaccine) 5 doses - 0 dose at birth, three primary doses at 6, 10 and 14 weeks and one booster dose at 16 -24 months of age.

3. Hepatitis B vaccine 4 doses - 0 dose within 24 hours of birth and three doses at 6, 10 and 14 weeks of age.

4. DPT (Diphtheria, Pertussis and Tetanus Toxoid) 5 doses - Three primary doses at 6, 10, 14 weeks and two booster doses at 16-24 months and 5 Years of age.

5. Measles (MMR vaccine) 2 doses - first dose at 9-12 months and second dose at 16-24 months of age.

6. TT (Tetanus) - 2 doses at 10 years and 16 years of age.

Booster dose: An additional dose of a vaccine is needed periodically to boost the immune system. Sometimes, the memory cells may lose their function - hence periodically a booster dose is given. For example, a booster shot of the tetanus and diphtheria vaccine is recommended for adults every 10 years. Booster dose is recommended only after the exposure to antigen. Booster doses are also given to prevent polio.

Introduction to Disinfectants

Disinfectants are mainly used to destroy the bacteria on inanimate objects, and can be of bacteriostatic or bactericidal. The dynamics of disinfectants i.e. action of disinfectants with time is high initially, and decreases with time following first order rate kinetics. The more the number of cells, the more the amount of time required.

The parameters that influence the activity of disinfectants are environment, more number of organisms, time, type of microorganism, temperature (at high temperatures the organisms can be destroyed easily), pH, concentration of the agent and physiological states like spore form, vegetative form or dry form.

Introduction to Diagnostic Tests

Diagnostic test is a procedure of examining, and confirming the presence of any disease in a suspected patient. There are different types of diagnostic tests among which identification of organism present in the patient through culture is a time consuming process. Another method adopted is identification of antigen or antibody. This antigen – antibody reaction tests are called as serological tests. The serological tests are direct and indirect tests. In direct serological test, the organism i.e., antigen is identified when reacted with a specific antibody. Indirect test is used to identify the kind of antibody is present by taking a known antigen. Different types of antigen - antibody tests are performed to diagnose the organism and disease like agglutination test, precipitation test, neutralization test, complement fixation test, immunofluorescence test, and radio immuno assays etc.

Other diagnostic tests includes; Skin tests (Schick's test), Delayed type of hypersensitivity test (Tuberculin or Mantoux peripheral smear test), Allergic tests (patch test or prick test).

Schick's Test

Schick's test is a method for determining the susceptibility to diphtheria which is an acute infectious and serious contagious disease caused by the bacillus, Corynebacterium diphtheriae. Schick's test is an antigen – antibody reaction test. The test is introduced by a scientist, Bela Schick. The procedure for Schick's test is - a very minute amount of the toxin of about 0.1 ml is taken and injected intradermally to the fore arm of the patient. And similarly an inactivated toxoid which is heated at 70°C for 30 minutes is injected to another forearm of the patient taken as control. If the skin around the injected area becomes red and swollen, indicates a positive result because the person lacks antibodies against the toxin and hence is susceptible to the disease and the control arm shown no reaction. The reaction is observed on 1^{st}, 4^{th} and on 7^{th} day. If the skin around the injected area shows no redness or swelling on both the arms - it indicates the negative test. In some cases, a pseudo-positive test can be observed where a red coloured inflammation is observed within 24 hours after injection on both the arms and it disappears within 4 days. Sometimes a combined reaction is observed, in which initially it looks like pseudo reaction where a red coloured inflammation is observed and disappears within 4 days but only on the control arm. This person is indicated as susceptible and hypersensitive to diphtheria. This test is not in practice now a days, as the children have been immunized earlier with the toxoid.

Delayed Type of Hypersensitivity Test (Mantaux Peripheral Smear)

Delayed hypersensitivity test or Mantoux test is used to diagnose tuberculosis and leprosy diseases caused by the bacteria Mycobacterium tuberculosis and Mycobacterium leprae respectively. If any part of the body is infected, it stimulates the T- cells which can be detected by “Delayed hypersensitivity test” or “Mantoux peripheral smear test”. In this test, a small amount of mycobacterial antigen (tuberculin) is injected intradermally - it sensitises T-cells - these T-cells activates macrophages and cause inflammation on the injected area. Tuberculin is the glycerol extract of bacillus but now a days purified protein derivative (PPD) is used in this method. About 5 Units i.e., 0.1 ml tuberculin is injected intradermally which produce the delayed hypersensitivity reaction after 78 hours. Measure the diameter of red induration. If a person have an induration of 5 to 15 mm or greater, it is interpreted as a positive test. If the induration is less than 2 mm or no induration gives negative test. Sometimes a false positive test is observed - it may be due to infection of non tuberculosis Mycobacteria or by previous vaccination of BCG. Hence, here symptoms of tuberculosis must also be considered. Sometimes, a false negative test is observed in the immunodeficient persons.

Agglutination Test (Widal Test)

Agglutination test or Widal test is a test which detects the presence typhoid and paratyphoid fever. The patient's serum is tested for O and H antibodies (agglutinins) against S. typhi 'O' antigen, H antigen, S. Paratyphi 'AO' antigen, 'AH' antigen, 'BO' antigen, 'BH' antigen etc. "O" antigen is a somatic antigen and "H" antigen is flagellar antigen. The diagnosis can take time - O agglutinins can usually be detected 6–8 days after the onset of fever and H agglutinins after 10–12 days. It is preferable to test two specimens of sera at an interval of 7 to 10 days. The method used is tube agglutination test. The patient's serum is collected and dilution of 1:20 is done by adding 0.1 ml of serum with 0.9 ml of saline. From this 0.5 ml is diluted with 0.5 ml of saline and 0.5 ml of specific antigen is added resulting in 1:40 dilution. And then from preceding tube 0.5 ml is diluted with 0.5 ml of saline and 0.5 ml of antigen is added resulting in 1:80 dilution. And from the resulted solution, 0.5 ml is diluted with 0.5 ml of saline and 0.5 ml of antigen is added leading to 1:160 dilution. Similarly the dilutions are prepared up to 1:1280. All the tubes are mixed well and kept in water bath at 37°C for 16-18 hours and observed for agglutination.

The titre of the patient serum using widal test antigen suspensions is the highest dilution of the serum sample that gives a visible red agglutination. Agglutination in the dilutions 1:80 and 1:40 are taken as negative. The titre value above 160 is considered as positive result and the value is above 320 is confirmatory test for typhoid. If S. typhi O-antigen is more than 1:80 and H-antigen is more than 160 - it is a confirmation of typhoid fever. The Widal test has some limitations – It is a time consuming test; may be falsely positive in patients who have had previous vaccination or infection with S. typhi; false negative results may be associated with early treatment, and may be due to antibody responses as well. Nowadays, slide widal test is done in which a drop of serum is placed on the reaction circle of slide. And a drop of antigen is added which shows agglutinations. The test is repeated with further semi quantitative dilutions.

Quantitative Buffy Coat Test (QBC)

QBC is an effective test for diagnosing blood parasites that cause malaria. It is based on acridine orange staining of centrifuged peripheral blood samples in a QBC tube and examination under UV light source (fluorescence microscopy). The acridine orange stain binds to the nucleus and cytoplasm of malarial parasite and emits green and red fluorescence when excited by blue light (at 460 nm) allowing the detection and examination of parasite morphology by fluorescence microscopy.

Procedure: A QBC tube (capillary tube) which is coated with acridine orange dye is taken and filled with 50-60 µl of blood and then closed. A close fitting plastic float which also contains blood is inserted inside the acridine orange-coated capillary tube. Now, the QBC tube is centrifuged at a speed of 12,000 rotations per minute for 5 minutes. With this the blood components get separated based on the density forming a buffy coat. Now the tube is observed under standard white light having UV microscope adaptor. The fluorescent malarial parasites are seen in different layers of the tube. Gametocytes will appear near the interface of the lymphocyte/monocyte and platelet layers. Schizonts and mature trophozoites may appear in the granulocyte layer. Ring-shaped immature trophozoites will appear throughout the red blood cell layer, with a concentration near the interface with the granulocyte layer.

Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. In ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme that can convert to some detectable signal. The enzyme labelled immunoglobulins develops the signals due to the hydrolysing enzymes which have a chromogenic substrate which will give a colour or reaction which can be observed by using microplate reader having 96 wells. The ELISA has been used as a diagnostic tool for detecting hepatitis and HIV and also has applications in food industry. The ELISA can be divided as competitive ELISA and non-competitive ELISA. The non-competitive ELISA is again classified as direct, sandwich, and indirect methods.

- The competitive ELISA – It is a competitive binding process executed by sample antigen and another added antigen. In this method, the antibody is coated on the microplate. Now the enzyme labelled as well as unlabelled antigens are added. Now the antigens will compete for binding to the specific antibody. If unlabelled antigens are present in high concentration, the less amount of labelled antigen forms complex with the antibody. When a particular substrate is added, the labelled antigen antibody complex will show a reaction which can be determined. For competitive ELISA, the higher the sample antigen (unlabelled) concentration, the weaker the eventual signal.

- Direct ELISA - This is the simplest type of ELISA in which, the antigen is adsorbed to a plastic plate, then an excess of another protein (normally bovine serum albumin) is added to block all the other binding sites. Now the enzyme linked antibody is added which adsorbs the antigen. Then a specific substrate is added which reacts with the enzyme and can be determined with a colour change.

- Sandwich ELISA – It identifies the antigens between two layers of antibodies. Hence named as 'Sandwich'. In this method, the plate is coated with antibodies and antigenic sample is added which forms antigen-antibody complexes. Now enzyme linked antibody is added which will bind to the antigen i.e., the Ag is stuck between two antibodies. Then a specific substrate is added which binds to the enzyme and gives a reaction which can be detected by a colour or fluorescent or electrochemical signal.

- Indirect ELISA – In this method, the antigen is adsorbed to a plastic plate, then an excess of another protein (normally bovine serum albumin) is added to block all the other binding sites. Now the antibody is added which adsorbs the antigen. Then, a secondary antibody (anti antibody) linked to enzyme is added to the plate which detects the antibody that is adsorbed to the antigen. Then the specific substrate is added which reacts with the enzyme and thus the antigen is determined.

Molecular Biological Techniques (PCR, Blotting Tests)

Molecular biological techniques detect specific sequences in DNA or RNA that may or may not be associated with disease. Nucleic acid–based testing is a modern technique which has become a crucial diagnostic tool not only in the identification of inherited genetic diseases but also a wide variety of infectious diseases. Molecular biological techniques include PCR and Blotting techniques. The DNA can be separated by electrophoresis, probes or by hybridization techniques. In electrophoresis, the DNA, RNA, or proteins are first digested with endonucleases and then separated in a gel or fluid under the influence of an electric field. Then a probe which is a specific radioactive labelled DNA or RNA fragment used to detect the DNA or RNA or protein is introduced in the sample and hybridized. Wherever hybridization occurs, a DNA or RNA molecule or protein can be identified.

PCR (Polymerase Chain Reaction): PCR is a method used to amplify (make many more identical copies) the DNA or RNA unique sequences so they can be used to determine source of a pathogenic organism in the sample. Kary Mullis first introduced the PCR technique and was awarded the Nobel prize. The reagents or chemicals needed for this test are - a sample that contains a nucleotide sequence, DNA primers: short single stranded DNA that attaches to nucleotide sequences that promotes synthesis of a complementary strand of nucleotides, DNA polymerase, nucleotides and a cofactor Magnesium. The procedure - first step is denaturation of double stranded DNA at a temperature of 90° C for one minute which separates the two DNA strands. The second step is annealing at 54°C temperature where the DNA primers and DNA polymerase bind to individual single stranded DNA. The DNA polymerase uses them to make new complementary strands of DNA and this process is more rapid at 72°C at which normal polymerase will not be able to act hence a specialized DNA polymerase called Taq polymerase (obtained from a bacteria - Thermus aquaticus) is used. The extensions are done by the nucleotides, the building blocks. Magnesium ions are also used as co-factor in this process. A single short segment of DNA can be amplified to about 100 billion copies after 40 doubling cycles. PCR tests can detect and identify pathogenic organisms in patient. For example - HIV and other viruses and certain fungi. PCR tests are also used to identify and characterize genetic mutations, genetic fingerprinting, forensic investigations etc.

Blotting techniques: Another molecular biological technique is blotting techniques which include – southern blotting techniques, northern blotting techniques, and western blotting techniques. Southern blotting technique is first developed by Edward Southern, which is widely used to detect DNA fragments in the sample. Northern blotting technique is used to detect specific RNA sequences. Western blotting technique is used to detect certain proteins and other molecules.

- In southern blotting technique, the DNA is fragmented and separated by digestion by using restriction endonucleases followed by gel electrophoresis. The DNA fragment is blotted on the nitrocellulose membrane. Then a probe which is a specific radioactive labelled DNA or RNA fragment used to detect the DNA or RNA or protein and hybridized. Wash the excess of probe bound non-specifically to the membrane and the hybridized radioactive probes are detected by using autoradiography. Southern blotting technique has various applications like to identify specific DNA in a DNA sample, to diagnose various infectious diseases, to identify genetic mutations, in gene mapping etc.

- Northern blotting technique is similar to southern blotting technique, but instead of DNA, RNA is used. The RNA is denatured, blotted followed by treated with probes like mRNA or RNA with DNA etc. Then hybridized probes are identified with autoradiography. This technique is used to determine the transcription process.

- Western blotting is a widely used technique for the detection and analysis of proteins based on their ability to bind to specific antibodies. In this process the protein sample is digested with certain reagents or buffers which denature the protein. The fragmented protein is subjected to sodium dodecyl Sulfate (SDS) gel electrophoresis followed by blotting which is done on a nitrocellulose membrane. Then the primary antibody is added to the membrane on which proteins are blotted. After a wash is conducted to remove unbound primary antibody, secondary antibody usually which is conjugated with an enzyme such as HRP (Horse Radish Peroxidase) is added and the secondary antibody recognizes the primary antibody. A chemiluminescent substrate is added which binds to the enzyme. The protein is detected which is dependent upon the enzyme to which the secondary antibody is conjugated. Once the substrate has been added, a colour change or a light is being emitted that can be detected with film or a photo imager. This technique is used for protein analysis, mapping of proteins, identifications of toxins, and infectious diseases.

Life Cycle of a Malarial Parasite

Malaria is a mosquito borne disease; transmitted through female anopheles mosquito. The causative agent is a parasite called Plasmodium which is a protozoal parasite. These are heterotrophs. The different species of plasmodium that causes malaria are - Plasmodium falciparum, P. vivax, P. ovale, P. malariae.

Life cycle: Plasmodium requires two hosts in its life cycle. The primary host is female anopheles mosquito (sporogony) and the secondary host is human (schizogony). In human, the life cycle is divided into exo-erythrocytic cycle and erythrocytic cycle. The life cycle of plasmodium starts with sporozoite stage. When the mosquito bites a healthy person, the sporozoites present in the saliva of mosquito enters into the bloodstream and then enters into the liver. In the liver cells, the sporozoites multiply and this asexual division in the liver is called schizogony leading to formation of schizonts. The erythrocytic cycle starts after schizogony - in this, the schizonts enter the red blood cells and transform into mature schizonts. These mature schizonts get converted to merozoites and merozoites are transformed to trophozoites. These trophozoites infect another red blood cell and forms schizonts and the cycle continues. After few cycles the merozoites gets converted to gametocytes. When the female anopheles mosquito bites an infected person, the gametocytes enter into the gut of the mosquito and leads to the formation of male and gametes. The fusion of male and female gametes leads to formation of a zygote. This cycle is called sporogony. The zygote gets transformed into motile ookinete which can be embedded into the exterior gut membrane and develops into an oocyst. Oocysts divide many times to produce large numbers of about 10,000 sporozoites. These sporozoites migrate to the salivary glands of the mosquito where they can be injected into the blood of the healthy human and the cycle repeats. The life cycle of plasmodium in mosquito is called Ross cycle.

Symptoms of malaria - shivering and chills, fluctuations in body temperature. If treatment has not started immediately, the symptoms will reappear on every third day. Based on the causes of recurrence of malaria, it is divided into three types - recrudescence malaria, relapse malaria, and re-infection.

Introduction to Microbial Culture Sensitivity Testing (Interpretation of Results)

Microbiology is the study of living organisms that are invisible to the naked eye - such as bacteria, virus, and fungi. In microbiological test - microbial organisms are let to reproduce in predetermined culture media under controlled laboratory conditions. Microbial culture is used to determine the type of organism and its abundance in the sample being tested or both. A laboratory test is performed to check the effectiveness of a drug against microorganisms. This is used to select the best effective drug regimen. The purpose of culture sensitivity test is to guide the clinician in selecting the best drug for an individual patient, to control use of inappropriate drug in clinical practice, to reveal changing trends in the local isolate, to overcome the microbial drug resistance. Sensitivity analysis starts with a bacterial sample. Culture is taken from blood, urine sputum and a wound. Selection of the appropriate method will depend on the intended degree of bioavailability of sources, availability of expertise and cost steps in analysis.

Minimum inhibitory concentration in microbiology is the lowest concentration of an antimicrobial that will inhibit the visible growth of microorganisms after overnight incubation. Minimum inhibitory concentration is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent. Agar dilution method follows principle of establishing the lowest concentration of the serially diluted antibiotic concentration at which bacterial growth is still inhibited. Antibiotics are diluted to various dilutions to test the MIC.

The sample is swabbed onto the growth media. A growth medium usually Mueller- Hinton agar is first even seeded throughout the plate with isolate of interest that has been diluted at standard concentration. Then using a dispenser such as the one pictured, antibiotic impregnated disc is placed on the agar surface. The test antibiotic immediately begins to diffuse outward from the discs, creating a gradient of antibiotic concentration. Most automated antimicrobial susceptibility testing systems provide automated inoculation, reading and interpretation.

Principles & Methods of Microbiological Assays

The agar diffusion test (Kirby–Bayer antibiotic testing, KB testing, or disc diffusion antibiotic sensitivity testing) is a test of the antibiotic sensitivity of bacteria. It uses antibiotic-impregnated wafers to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition. The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone, because a lower concentration of the antibiotic is enough to stop growth.

The bacteria in question are swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion is used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacterium. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection. MIC method which is also called serial dilution.

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that prevents visible growth of a bacterium. The MIC of a chemical is determined by preparing solutions of the chemical at increasing concentrations, incubating the solutions with the separate batches of cultured bacteria, and measuring the results using agar dilution or broth micro dilution. In medicine, culturing the organism infecting a patient with available antibiotic drugs and determining the MICs, is important for identifying the correct drug to actually give to the patient. The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by sub culturing to agar plates that do not contain the test agent. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculums. The MBC is complementary to the MIC whereas the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth; the MBC demonstrates the lowest level of antimicrobial agent that results in microbial death.

E Test, (Epsilometer test) is a 'ready-to-use' reagent strip with a predefined gradient of antibiotic for the determination of precise MIC values of a wide range of antimicrobial agents against different organism groups. When Etest is applied to the surface of an agar plate inoculated with the test strain, there is an instantaneous release of the antimicrobial gradient from the plastic carrier to the agar to form a stable and continuous gradient beneath and in the immediate vicinity of the strip. Etest incubation and reading times have been determined based on the intrinsic growth characteristics of the organism, and the specific incubation conditions. Therefore, for reliable and reproducible results, the stability of the gradient must be maintained for many hours. The predefined Etest gradient remains stable for at least 18 to 24 hours; that is, a period that covers the critical times of many species of fastidious and nonfastidious organisms. The bacterial growth becomes visible after incubation and a symmetrical inhibition ellipse centred along the strip is seen.

Microbiological Assay of Penicillin and Streptomycin

The microbiological assay of an antibiotic is based upon comparison of the inhibition of growth of microorganisms by measured concentrations of the antibiotics under examination with that produced by known concentrations of a standard preparation of the antibiotic having a known activity. Two general methods are usually employed, the cylinder-plate (or cup-plate) method and the turbidimetric (or tube assay) method.

The cylinder-plate method depends upon diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a petri dish or plate to an extent such that growth of the added micro-organism is prevented entirely in a zone around the cylinder containing a solution of the antibiotic. The turbidimetric method depends upon the inhibition of growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favourable to its rapid growth in the absence of the antibiotic.

To prepare a stock solution, a quantity of the standard preparation of a given antibiotic is accurately weighed and dissolved in the solvent specified and then diluted to the required concentration as indicated. The solution to be stored in a refrigerator and used within the period of time. On the day of assay, from the stock solution five or more test dilutions, the successive solutions increasing stepwise in concentration, usually in the ratio 1:1.25 are prepared for cup plate method or in smaller ratio for tube assay. Use the final diluents specified and a sequence such that the middle or median has the concentration specified.

The test organism for each antibiotic is listed together with its identification number in the American Type Culture Collection (ATCC). The culture is maintained on slants of the medium and under the incubation conditions specified and transferred weekly to fresh slants.

Ex: Penicillin (antibiotic) - Staphylococcus aureus (test organism) – 29737 (ATCC No), Streptomycin – Klebsiella pneumoniae – 10031.

The media required for the preparation of test organism inocula is prepared from the ingredients. Minor modifications of the individual ingredients may be made, or reconstituted dehydrated media may be used provided the resulting media have equal or better growth-promoting properties and give a similar standard curve response.

The ingredients are dissolved in sufficient water to produce 1000 ml and sufficient 1 M sodium hydroxide or 1 M hydrochloric acid is added as required to adjust the pH.

Turbidimetric method - A method has been developed for turbidimetric measurement of bacterial growth in standard inexpensive test tubes with closures in-place. Liquid cultures and agar plug diffusion cultures can be assayed using an unmodified spectrophotometer. Growth curves of replicate cultures grown in test tubes are reproducible with respect to similarity of curve shape, onset of logarithmic growth phase, and maximum growth.

Microbiological Assay of Vitamin B2

Vitamin B_2 (Riboflavin) is an important component and essentially used in metabolism. Riboflavin deficiency results in stomatitis including red tongue with sore throat and inflammation of the corners of the mouth. Microbiological assay of vitamin B_2 is performed with the aid of microorganisms. Many therapeutic agents which either inhibit the growth of microorganisms or are essential for growth are standardized by microbial assays.

Principle: The main principle involved in the microbiological assay of vitamin B_2 includes mainly growth sensitivity in which known concentration of vitamin B_2 is compared with the test preparation. The main basis of this assay is to measure the ability of test organism to utilize the substance being assayed under nutritional conditions. The growth of test organism is proportional to the dose added to the medium.

Test organism: Selection of test organism is very important. In this assay **"Lactobacillus Rhamnosus"** is used. It is one of the fastidious organism and it requires elaborative nutrients. This organism is sensitive towards vitamin B_2. Even a small fluctuation in the quantity of vitamin B_2 will lead to a fluctuation in the growth of microorganism.

Media: The media used for this assay should contain all essential nutrients which helps for the growth of microorganism. As it is a fastidious organism it cannot synthesize nutrients by its own. So all the essential nutrients should be provided in the media or lyophilized cells can be used for conduction of a particular organism. Assay media contains all the ingredients except vitamin B_2. pH of this media is 6.1 ± 0.2 at 25℃. The media is autoclaved at 121℃ for 15-20 min and then cooled.

Method: The prepared media is cooled and transferred into test tubes. Then 10ml of media is transferred to 10 test tubes aseptically. The test tubes should be sterilized and cooled. Then different vitamin B_2 concentrations should be transferred into corresponding tubes (the corresponding concentrations of vitamin B_2 in 10 test tubes is 0.00, 0.025, 0.05, ...0.25 nanograms). The test vitamin B2 solution is diluted as of standard and added in one of the tubes as vitamin B_2 supplement. In each test tube 100µl of test organism is incorporated. The test tubes are incubated at 37℃ for 24-48 hrs. After incubation for a particular period, the growth of organism is observed. The growth of microorganism is measured by optical density at 520nm. There is maximum absorption at a particular wavelength. All the test tubes are measured by optical density and so is the test sample.

Standard Curve: After observing optical density for all the samples, the graph is plotted. The standard curve is plotted by taking concentration of vitamin B_2 on x-axis and observances on y-axis. At the beginning of concentrations, the growth is less and as the concentrations increases the growth also increases and so do the observances. The straight line is obtained and from this line, the concentration of unknown is measured. After extrapolation on x-axis the concentration of test is obtained.

This method is known as turbidimetric method because turbidimetry is also measured in a liquid media. An alternate method is also present i.e agar diffusion method and it is also called as cup plate method.

Cup Plate Method: The vitamin B_2 assay is alternatively done by this method. A petri plate consists of agar media (solid media which contains all nutrients and agar -solidifying agent) except vitamin B_2. The agar media is autoclaved, cooled and transferred to petri plates and solidified in gel form. To this agar media the test organism (lactobacillus rhamnosus) is transferred into surface of this media and spreaded with L-shaped glass rod. After application of test organism cups are made by appropriate diameter (6-8mm) by using a borer. The various vitamin B_2 concentrations are prepared and added to each cup from lower to higher concentrations. One of the cups contains unknown concentration. The cups are kept aside so that vitamin B_2 diffuses into the media. The plates are incubated in incubator at 37 ℃ for 24-48 hrs. After incubation period, the growth is observed around the cups because test organism is sensitive to vitamin B_2. The zone of growth(mm) is observed around the cups. After observing all the results the standard curve is plotted by taking concentration of vitamin B_2 on x-axis and zone of growth(mm) on y-axis. The graph is extrapolated to know the unknown concentration.

Microbiological Assay of Vitamin B12

Vitamin B12 is also called as cyanocobalamin; it helps in growth of microorganisms. It has been demonstrated that lactobacillus lactis dorner (ATCC 8000) must be cultivated with special precautions to prevent disassociation when used in the microbiological assay of vitamin B12. lactobacillus leichmannii has been found to require nutrients similar to those needed by lactobacillus lactis dorner and to exhibit little tendency to disassociate. The use of a stable test organism in the microbiological assay of vitamin Bl2 would be of distinct advantage.

The method herein outlined employs lactobacillus leichmannii (ATCC 4797) as the test organism. The medium for the assay is a dehydrated product prepared by the Difco Laboratories. The standard and test materials are diluted to a concentration of 0.02 y per ml. in distilled water. The test organism responds most satisfactorily within the range of 0.01 to 0.10 y per tube levels. Duplicate or triplicate tubes on each level give growth response in good agreement turbidimetrically. Tubes containing only distilled water and the basal medium produce no growth in the absence of vitamin B12. The results of the assay are evaluated turbidimetrically after 18 hours growth at 37. Inoculums' for the test is prepared by washing four or five times in sterile saline; the cells produced from a 24 hour growth of Lactobacillus leichmannii in Difco micro inoculum broth.

Standardisation of Vaccines and Sera

Standardisation can also be called as quality control of vaccines and sera. Comparison of test sample assay with the standard is also called as standardisation. Vaccines provide an antigenic stimulus that does not cause disease but can produce long lasting, protective immunity sera containing antibodies. Toxicity test - Preventative and therapeutic vaccines are increasingly used during pregnancy - special considerations are necessary for development of toxicity testing.

Sterility test - It can be defined as the freedom from the presence of viable microorganisms. Most biological products, including vaccines, administered by the parenteral route are required to be tested for sterility at the final container and also at various stages during manufacture. The sterility testing as per Code of Federal Regulations and the United States Pharmacopoeia is based on the observation of turbidity in liquid culture media due to growth of potential contaminants.

Potency test - Combination vaccines differ from single-component vaccines in composition and manufacturing method and also poses significant challenges in implementing effective quality-control tests, including measurement of potency. Because each combination vaccine is unique, existing guidelines often fail to provide sufficient information to overcome the inevitable problems encountered when developing and implementing potency tests. The BCG albumin assay is designed to measure albumin directly without any pretreatment of samples, such as serum, plasma, urine, and biological preparations. It may also be used to measure effects of drugs and other compounds on albumin metabolism. It can be used for corvette or multiwall plate assays. The multiwall plate assay uses samples as small as 5 mL and can be readily automated as a high-throughput assay for thousands of samples per day. The procedure involves addition of a single working reagent and 5 minute incubation. The intensity of the color, measured at 620 nm, is directly proportional to the albumin concentration in the sample.

Safety test - The abnormal toxicity test (ATT) using mice and guinea pigs is a non-specific safety test for vaccines, sera and immunoglobulins. Positive ATT's never resulted from the insufficient quality of a batch. On the other hand, vaccines causing adverse reactions in the target species were not identified by the ATT.

Endotoxins test- Twenty samples of commercial vaccines intended for administration to humans were assayed for the presence of bacterial endotoxins by using the Limulus amebocyte lysate test. Sixteen of the vaccines contained more than 0.1 mg of endotoxins per ml. These results suggest that at some stage of preparation, the vaccines have contained varying amounts of gram-negative bacteria and may indicate the presence of other bacterial products as well.

Study of Typhoid

The causative agent of typhoid is a bacteria, Salmonella typhi belonging to family Enterobacteriaceae. The bacteria is a gram negative, rod shaped bacteria in single arrangement. It has perichitous flagella for motility, it is non spore forming, facultative anaerobic, and capsular organism.

Virulent factors: The bacteria causes the infection due to the following virulent factors - H antigen present on the flagella, O antigen is a somatic antigen present on the body surface of the organism, V_i capsular antigen which is a capsular producing antigen, endotoxin called lipopolysaccharide, secretory protein called invasin which enters the non-phagocytic cells of the host. Due to these virulent factors the bacteria can escape from the host's immune mechanism.

Mechanism of infection: Salmonella typhi enters the host through the contaminated food and water, and from the stomach it enters into the intestine. In the intestine it crosses the epithelium and reaches systemic circulation and enters into various tissues and organs.

Signs and symptoms: In the first week of infection fluctuating bradycardia, malaise, headache, cough, and abdominal pain were observed. In the second week of infection high fever, bradycardia, delirium, rose colour spots on chest and trunk region, abdominal pain, diarrhoea or constipation are observed. In the third week many complications like intestinal haemorrhage i.e., perforation mainly in the ileum region, where the organism enters the systemic circulation and cause various metastatic complications like abscesses in the liver, cholecystitis (inflammation of gall bladder), endocarditis (inflammation of endocardium), osteitis and also oscillating type of fever usually high in afternoon, dehydration, delirium, and decrease in the platelet count leading to internal bleeding.

Diagnosis: The diagnosis can be done by clinical symptoms, patient history, use of serological tests like Widal test in which the blood sample from the patient is taken and mixed with specific antibodies which react with H and O antigens of the bacteria present in the blood and forms antigen-antibody agglutination. It is a presumptive test. Other confirmation tests are isolation test in which the cultures from the blood sample are isolated by using Macconkey's agar/EMB agar media, and biochemical characterization (non lactose forming and non gas producing organism) are used. The advanced techniques like PCR and blot tests are also used.

Mode of transmission: The main source of transmission of typhoid is vomiting, fecal and urine disposals from the infected person. It may be transmitted directly or indirectly by carriers like flies. The transmission can be done by contaminated water and food, improper hygienic and sanitary conditions.

Immunization: The immunization can be done by vaccination. The V_i capsular antigen vaccine or killed bacterial vaccine is given by parenteral route. The immunity towards the infection may be for only 2 – 3 years.

Prevention and control: The typhoid infection can be prevented by practicing proper sanitation and hygienic life style, careful food preparations, controlling flies, water purification, complete treatment for the infected persons, and educating the public about the disease.

Treatment: The first choice of the drug is fluoroquinolones e.g. ciprofloxacin and third generation cephalosporins e.g. ceftriaxone, cefotaxime, and cefixime. Other antibiotics used are ampicillin, chloramphenicol and combination of trimethoprim-sulfamethoxazole. If complete drug therapy is not implemented, then the organism may get drug resistance and becomes difficult to remove the infection completely.

Study of Tuberculosis

The causative agent of tuberculosis is Mycobacterium tuberculosis belonging to the family Mycobacteriaceae. The other strains that cause tuberculosis are M. bovis, M. africanum, M. carnetti, M. microti etc. The morphological features of the bacteria are that it is a small, straight, slender rod shaped, non-motile, non-capsulated, non-spore forming, aerobic organism. The presence of mycolic acids in the cell wall is a characteristic feature due to which the bacteria gets resistance towards various antibiotics and disinfectants, and escapes from the phagocytic mechanism of the host. The bacteria is identified by the acid fast staining as it does not take up the normal stains due to the presence of mycolic acids in the cell wall. Thus it is differentiated as acid fast positive bacteria.

Pathophysiology: Mycobacterium tuberculosis enters the respiratory tract through the nasal route and then enters the alveoli. Due to the presence of mycolic acids in the cell wall, the bacteria escapes from the phagocytic action of the alveoli. But the other immune cells like fibroblasts, cytotoxic T cells, and macrophage lines deploy around the bacterium. This deployment of bacterium is called as tubercle. The tubercle is very minute in size which may not be noticed. When the immunity of the host becomes weak and the immune cells are unable to control the bacterium, this bacterium starts to multiply and the tubercle grows in larger size. The continuous multiplication of bacteria within the tubercle leads to the rupture of alveoli and affects the respiratory system wherein the bacteria enters the circulatory system. Through the systemic circulation the bacteria reaches different tissues and organs by forming a granuloma. The infection within the respiratory tract is called pulmonary infection and the infection within the systemic circulation is called extra pulmonary tuberculosis.

Signs and symptoms: The general symptoms are fever, chills, night sweats, loss of appetite, severe fatigue, persistent and worsening cough. The pulmonary signs are chest pain, cough with sputum, sometimes blood in the sputum is noticed due to rupture of alveoli. The extrapulmonary signs are observed depending on the organ which is affected by the bacteria. TB pleurisy, CNS - meningitis, lymph - scrofula in neck, bones and joints - pott spine, liver - hepatitis, skin - ulcer, and genito urinary infection. If the tuberculosis is widespread throughout the body it is termed as miliary tuberculosis.

Diagnosis: If the symptoms persist over a period of two to three weeks, it is called active tuberculosis. This can be diagnosed by chest x - ray, multiple sputum culture by acid fast staining technique, biopsy cultures of infected tissue or organ, PCR, adenosine deaminase test etc. Latent tuberculosis is which wherein no clinical symptoms were observed. It is diagnosed by Mantoux tuberculin skin test, and advanced tests like α - gold test or ELISA.

Mode of transmission: The major route of transmission of tuberculosis is aerial route i.e. through the droplets of cough and sputum from the infected person. Other routes of transmission are sharing fomites or objects, and contaminated food.

Immunization: The immunization of tuberculosis was done through a vaccine known as BCG (Bacillus Calmette Guerin) vaccine, which is a successful live attenuated vaccine.

Prevention and control: The tuberculosis can be prevented by proper vaccination to infants, proper diagnosis and medical therapy, avoiding aerial route of transmission by using masks, proper diet and hygienic life, and educating the public.

Treatment: Treatment for the tuberculosis can be done by use of certain antibiotics like isoniazid, ethambutol, rifampicin and pyrazinamide for prolonged period of time i.e. for about 6 months. The multi drug therapy is used in order to avoid the bacterial drug resistance.

Study of Malaria

Malaria is a mosquito borne disease transmitted through female anopheles mosquito. The causative agent is a parasite known as Plasmodium which is a protozoal parasite. The different species of Plasmodium that causes malaria include Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. Among these species P. falciparum and vivax are majorly disease causing organisms. P. ovale and P. malariae show less occurrence of malaria and P .knowlesi is a zoonotic species (cause malaria in animals).

Signs and symptoms: signs and symptoms can be observed within 8-25 days after the infection.

- Initial non-specific symptoms are flu, malaise, fever, headache, body pains, nausea, and vomiting.
- The other nonspecific symptoms are haemolytic anaemia, jaundice, haemoglobin in urine, retinal damage and convulsions.
- The classical symptoms include cyclical occurrence of sudden cold, fever, severe sweating and shivering.

During infection with vivax and ovale, the cyclical responses are seen every two days (tertian fever). Infection due to falciparum is dangerous as the cyclical responses are seen for every 36-48 hours with fever. Sometimes the organism enters the cerebral region causing cerebral malaria, wherein the neurological symptoms are seen in patients which include abnormal posturing, nystagmus, seizures, and coma. Various complications due to classical symptoms of malaria are respiratory distress, metabolic acidosis, pulmonary oedema, pneumonia, anaemia, cerebral malaria leading to splenomegaly (enlargement of spleen), hepatomegaly (inflammation of liver) and hypoglycaemia.

Life cycle of plasmodium: The life cycle of plasmodium starts with sporozoite stage, when the mosquito bites a healthy person the sporozoites present in the saliva of mosquito enters into the bloodstream and then into the liver. In the liver cells the sporozoites multiply asexually developing merozoites. The merozoites rupture the liver cells and enters the bloodstream again. In the RBCs the merozoites are transformed into trophozoites and schizonts, and mature into merozoites again. These merozoites enters the liver or attacks the adjacent RBCs, wherein some merozoites are transformed into the sexual forms. The cyclic process that occurs in liver is known as exo-erythrocytic cycle and that occurs in RBCs is known as erythrocytic cycle. When a mosquito bites the infected person the sexual forms enters the mosquito. As this life cycle continues the classical symptoms like flu, cold, fever and shivering may appear frequently.

Recurrent malaria: Based on the cause of recurrence of malaria it is divided into three types.

- Recrudescence malaria - even after the anti-malarial therapy some of the parasites may survive in the blood which leads to the recurrence of malaria.
- Relapse malaria - for a considerable period of time the parasite undergoes a dormant stage called Hypnozoites. When the person becomes healthy the hypnozoites gets activated as merozoites and leads to the recurrence of malaria.
- Reinfection - the person after the complete therapy becomes healthy. When an infected mosquito bites to this person it leads to a new infection to the person.

Resistance of malaria in certain genetic traits: Genetic factors that influence the resistance towards the malaria are:

- Sickle cell traits - the normal shape of RBCs is disc whereas in individuals with sickle cell trait the RBC are sickle shaped
- Thalassemia traits - abnormality in the haemoglobin
- Glucose-6-phosphate deficiency trait - deficiency in the glucose-6-phosphatase enzyme responsible for the glucose uptake
- Absence of Duffy antigens trait - antigens are the surface receptors with the help of which the merozoites enters the RBCs. All these genetic abnormalities inhibit the entry of merozoites into the RBC's.

Diagnosis: It includes patient history, spleen enlargement, low platelet count, increased bilirubin levels, blood smear test, and buffy coat test (a nonspecific test), antigen serological test, and PCR (polymerase chain reaction) tests are specific tests for malaria.

Prevention: Malaria can be prevented by prophylactic medication, mosquito eradication or by prevention of mosquito bites (use of mosquito repellents, mosquito nets, creams, sprays etc). Prevention of malaria in community basis can be done by health education, covering the areas of stagnant water, spraying chemicals on drainage areas, and giving proper preventive therapy to children and pregnant women.

Treatment: Malaria can be treated with

- Antiprotozoals - chloroquine, mefloquine etc.
- Antibiotics - doxycycline, atovaquone and proguanil hydrochloride combination, amodiaquine, lumefantrine, sulfadoxine, pyrimethamine, combination of artemisinin and piperaquine, for pregnant women quinine and clindamycin combination and artemisinin derivatives can be used.

Study of Cholera

The causative agent of cholera is the bacteria, Vibrio cholerae belonging to the family Vibrionaceae which causes infection to the small intestine. Two strains of Vibrio cholerae O_1 and O_{139} i.e., Eltor and Classical strains respectively are mainly causing the cholera. The morphological features of this bacteria are gram negative bacteria, comma shaped or bent rod shaped, single arrangement, contains single polar flagella for motility, non capsular, non spore forming, facultative anaerobic organism and can tolerate high pH up to 8. The susceptibility of this disease is more in children, individual with less immunity and malnourished persons.

Signs and symptoms: The primary symptoms are profuse diarrhoea and vomiting with complete loss of clear fluids. Other symptoms are severe dehydration with loss of electrolytic balance, fever in rare cases, systemic acidosis and decrease in blood pressure. Due to these symptoms the patient may look lethargic, experience dry mouth, decrease in skin texture and decrease in urine output, muscle cramps, seizures, altered consciousness, and coma (rarely).

Mode of transmission: The transmission is mainly through contaminated water and food. The main source of infection is discharge from the infected person and rarely through direct contact with the infected person or by the use of articles and due to carriers like flies.

Mechanism of infection: Vibrio cholerae through contaminated food and water enters the stomach and reaches the intestine. The bacteria gets multiplied in the intestinal epithelium and releases exotoxin called cholera enterotoxin. This exotoxin has two units A and B among which B unit is responsible for binding to the intestinal epithelial cells and A unit is responsible for the penetration into the epithelial cells leads to the accumulation of cAMP. This accumulation of cAMP leads to the absorption of excess of water and electrolytes into the intestine and leads to diarrhoea and vomiting. The bacteria also have an endotoxin called lipopolysaccharide which may also leads to the infection.

Diagnosis: The diagnosis can be done by observing the clinical symptoms like profuse diarrhoea or vomiting, by patient history, serological tests by collecting the fecal samples or rectal swabs, determination of bacteria on selective differential media and biological tests for Vibrio cholerae.

Prevention: Cholera can be prevented by-practicing proper sanitation, disposals or articles of patients are to be disinfected, proper sewage treatment, water purification by RO, UV, chlorination, boiling etc, controlling the flies and educating the people about the disease.

Vaccination: The vaccination for cholera can be given in the form of injection. The vaccine is the killed bacterial vaccine. The virulent strains of Vibrio cholerae are killed without disturbing their antigenic property. The immunity of the vaccine is confined for about 3-4 years.

Treatment: The treatment can be done with fluid supplements like electrolytes, bicarbonates, glucose, ORS and intravenous fluid injections. The antibiotic therapy includes doxycycline for 1-3 days and other antibiotics are erythromycin, tetracycline, chloramphenicol and fluoroquinolones.

Study of Hepatitis

Hepatitis is a medical condition defined as the inflammation of the liver. The condition is self-limiting, can progress to fibrosis and cirrhosis. Hepatitis may occur with limited or no symptoms, but it often leads to jaundice, poor appetite and malaise. Hepatitis is considered to be acute when it lasts less than six months and chronic when it persists longer.

Causes: Viral hepatitis is the most common cause of hepatitis worldwide. Other common causes of non-viral hepatitis include toxic and drug- induced alcoholic, autoimmune, fatty liver and metabolic disorders. Less commonly some bacterial, parasitic, fungal, mycobacterial and protozoal infections can cause hepatitis.

Viral hepatitis: The most common causes of viral hepatitis are the five unrelated hepatotropic viruses - Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D (which requires hepatitis B to cause disease), and Hepatitis E.

Hepatitis A virus (HAV) is present in the faeces of infected persons and is most often transmitted through consumption of contaminated water or food. Certain sex practices also spreads HAV. Infections are mild in many cases, with most people showing full recovery and remaining immune from further HAV infections. However, HAV infections can also be severe and life threatening. Most people in areas of the world with poor sanitation have been infected with this virus. Safe and effective vaccines are available to prevent HAV.

Hepatitis B virus (HBV) is transmitted through exposure to infective blood, semen, and other body fluids. HBV can be transmitted from infected mother to infant at the time of birth or from a family member to infant in early childhood. Transmission may also occur through transfusions of HBV-contaminated blood and blood products, contaminated injections during medical procedures, and through injection drug use. HBV also poses a risk to health care workers who sustain accidental needle stick injuries while caring for infected-HBV patients. Safe and effective vaccines are available to prevent HBV.

Hepatitis C virus (HCV) is mostly transmitted through exposure to infective blood. This may happen through transfusions of HCV-contaminated blood and blood products, contaminated injections during medical procedures, and through injection drug use. Sexual transmission is also possible, but is much less common. There is no vaccine for HCV.

Hepatitis D virus (HDV) infections occur only in those who are infected with HBV. The dual infection of HDV and HBV can result in a more serious disease and worsens the outcome. Hepatitis B vaccines provide protection from HDV infection.

Hepatitis E virus (HEV) is mostly transmitted through consumption of contaminated water or food. HEV is a common cause of hepatitis outbreaks in developing parts of the world and is increasingly recognized as an important cause of disease in developed countries. Safe and effective vaccines to prevent HEV infection have been developed but are not widely available.

Signs and symptoms:

Acute: Initial features are of nonspecific flu-like symptoms, common to almost all acute viral infections and may include malaise, muscle and joint aches, fever, nausea or vomiting, diarrhea, and headache. More specific symptoms, which can be present in acute hepatitis from any cause, are: profound loss of appetite, aversion to smoking among smokers, dark urine, yellowing of the eyes and skin and abdominal discomfort. Physical findings are usually minimal, apart from jaundice, tender enlargement of the liver, enlarged lymph nodes in 5%, and enlargement of the spleen. Acute viral hepatitis is more likely to be asymptomatic in children. Symptomatic individuals are present after a convalescent stage of 7 to 10 days, with the total illness lasting for weeks. A small proportion of people with acute hepatitis progress to acute liver failure, in which the liver is unable to remove harmful substances from the blood (leading to confusion and coma due to hepatic encephalopathy) and produce blood proteins (leading to peripheral edema and bleeding).

Chronic: Chronic hepatitis may cause nonspecific symptoms such as malaise, tiredness and weakness, and often leads to no symptoms at all. It is commonly identified on blood tests performed either for screening or to evaluate nonspecific symptoms. The presence of jaundice indicates advanced liver damage. On physical examination there may be enlargement of the liver. Extensive damage to and scarring of liver (i.e. cirrhosis) leads to weight loss, easy bruising and bleeding, peripheral edema(swelling of the legs) and accumulation of ascites (fluid in the abdomen). Eventually, cirrhosis may lead to various complications like esophageal varices (enlarged veins in the wall of the esophagus that can cause life-threatening bleeding), hepatic encephalopathy (confusion and coma) and hepatorenal syndrome (kidney dysfunction). Acne, abnormal menstruation, lung scarring, inflammation of the thyroid gland and kidneys may be present in women with autoimmune hepatitis.

Mechanism: The specific mechanism varies and depends on the underlying cause for the condition. In viral hepatitis, the presence of the virus in the liver cells causes the immune system to attack the liver, resulting in inflammation and impaired function. Some hepatitis, often including hepatitis caused by alcoholism, fat deposits accumulate in the liver, resulting in fatty liver disease, which is called as steatohepatitis.

Diagnosis: Diagnosis is made by assessing an individual's symptoms, physical examination, and medical history, in conjunction with blood tests, liver biopsy, and imaging. Blood testing includes blood chemistry, liver enzymes, serology and nucleic acid testing. Abnormalities in blood chemistry and enzyme results are indicative of certain etiologies or stages of hepatitis. Imaging can identify steatosis of the liver but liver biopsy is required to demonstrate fibrosis and cirrhosis. A biopsy is unnecessary if the clinical, laboratory, and radiologic data suggests cirrhosis. Furthermore, there is a small but significant risk to liver biopsy, and cirrhosis itself predisposes for complications caused by liver biopsy.

Viral hepatitis: Viral hepatitis is mostly diagnosed through clinical laboratory testing. Some of these tests react with the virus or parts of the virus, such as the Hepatitis B surface antigen test or nucleic acid tests. Many of the tests are serological that react to the antibodies formed by the immune system. For some major causes of viral hepatitis, such as Hepatitis B, there are multiple serological tests used that provide additional information for diagnosis.

Preventive Measures of Hepatitis:

- **Vaccines** : Vaccines are available to prevent hepatitis A and B. Hepatitis A immunity is achieved in 99-100% of persons receiving the two-dose inactivated virus vaccine. Vaccines to prevent Hepatitis B have been available since 1986 and have been incorporated into at least 177 national immunization programs for children. Immunity is achieved in greater than 95% of children and young adults receiving the three-dose recombinant virus vaccine. The W.H.O. recommends vaccination of all children, particularly newborns in countries where hepatitis B is common to prevent transmission.

Study of Meningitis

Meningitis is the inflammation of the protective membranes covering the brain and spinal cord, called as the meninges. Meningitis is usually caused by infection from viruses, bacteria, fungi, and parasites.

Bacterial - premature babies and newborns, are commonly infected by group B streptococci subtypes III which normally inhabit the vagina and those that normally inhabit the digestive tract are Escherichia coli. Older children are more commonly affected by Neisseria meningitides (meningococcus), Streptococcus pneumoniae (serotypes 6, 9, 14, 18 and 23) and those under five by Haemophilus influenzae type B. In adults, N. meningitidis and S. pneumoniae together cause 80% of all cases of bacterial meningitis.

Viral - viruses that cause meningitis include enteroviruses, herpes simplex virus type 2, varicella zoster virus (known for causing chickenpox and shingles), mumps virus, HIV, and LCMV.

Parasitic - the most common parasites causing meningitis are Angiostrongylus cantonensis and Gnathostoma spinigerum.

Signs and Symptoms - In adults, severe headache, nuchal rigidity (inability to flex the neck forward passively due to increased neck muscle tone and stiffness), the classic triad of diagnostic signs consists of nuchal rigidity, sudden high fever, and altered mental status. Other signs commonly associated with meningitis include photophobia (intolerance to bright light) and phonophobia (intolerance to loud noises). Small children often do not exhibit the aforementioned symptoms, and may only be irritable and looking unwell. In infants up to 6 months of age, bulging of the fontanelle (the soft spot on top of a baby's head) may be present. Other features that distinguish meningitis from less severe illnesses in young children are leg pain, cold extremities, and an abnormal skin color. The infection triggers sepsis, a systemic inflammatory response syndrome of falling blood pressure, fast heart rate, high or abnormally low temperature and rapid breathing. The inflammation of the meninges may lead to abnormalities of the cranial nerves. Visual symptoms and hearing loss may be present. Inflammation of the brain leads to weakness, loss of sensation, or abnormal movement or function of the part of the body supplied by the affected area in the brain.

Mechanism - In bacterial meningitis, bacteria reaches the meninges by one of the two main routes, through the bloodstream or through direct contact between the meninges and either the nasal cavity or the skin. In most cases, meningitis follows invasion of the bloodstream by organisms that live upon mucous surfaces. Once bacteria have entered the bloodstream, they enter the subarachnoid space in places where the blood brain barrier weak such as the choroid plexus. The inflammation that occurs in the subarachnoid space during meningitis is not a direct result of bacterial infection but can rather largely be attributed to the immune response.

Diagnosis: CSF findings such as glucose levels, protein levels and cells, blood tests and imaging - blood tests are performed for markers of inflammation, as well as blood cultures. Various more specialized tests may be used to distinguish between various types of meningitis. A latex agglutination test may be positive in meningitis caused by bacterial meningitis. PCR is a technique used to amplify small traces of bacterial DNA in order to detect the presence of bacterial or viral DNA in cerebrospinal fluid; it is a highly sensitive and specific test since only trace amounts of the infecting agent's DNA is required.

Prevention - For some causes of meningitis, prophylaxis can be provided in the long term with vaccine. E.g. immunization against Haemophilus influenzae type B in their routine childhood vaccination schemes; vaccination against Streptococcus pneumoniae; childhood vaccination with BCG. Short-term antibiotic prophylaxis is also a method of prevention, particularly of meningococcal meningitis. In cases of meningococcal meningitis, prophylactic treatment of close contacts with antibiotics can reduce their risk of contracting the condition.

Treatment

Initial treatment: Treatment with wide-spectrum antibiotics should not be delayed while confirmatory tests are being conducted. If meningococcal disease is suspected in primary care, guidelines recommend that benzylpenicillin be administered before admitting to hospital. IV fluids should be administered if hypotension (low blood pressure) or shock is present. Empiric antibiotics (treatment without exact diagnosis) must be started immediately, even before the results of the lumbar puncture and CSF analysis are known.

Viral meningitis: typically requires supportive therapy only; most viruses responsible for causing meningitis are not amenable to specific treatment. Mild cases of viral meningitis can be treated at home with conservative measures such as fluid, bedrest, and analgesics.

Fungal meningitis: Fungal meningitis, such as cryptococcal meningitis, is treated with long courses of highly dosed antifungals.

Study of Syphilis

Syphilis is a sexually transmitted infection caused by Treponema pallidum, a spirochete belonging to the family spirochaetaceae. It is a spiral shaped organism, very thin and long, cannot be observed under normal microscope. It is a gram negative bacteria, and is a motile organism containing periplasmic flagella. It is an anaerobic, non spore and non capsule forming organism. Syphilis is a chronic infection and based on the intensity of infection it is of two types acquired (through sex) and congenital (at birth).

In acquired syphilis treponema pallidum enters into the epithelial layer of urino-genital organs during intercourse, leading to the activation of immune system by the release of macrophagic lymphocytes forming a granuloma, which limits the disease to some extent. As the organism does not contain any antigens, it is not affected by the granuloma, but it slowly enters into the lymph and then into blood, and reaches liver, heart, joints, genital organs etc. In case of congenital syphilis, the mother is the infected person. The organism easily crosses placental barrier and reaches fetus, and affects different systems.

Signs and symptoms of acquired syphilis include primary, secondary, tertiary and latent stages. Primary syphilis involves development of chancre (granuloma formed by the accumulation of immune cells) on the genital organs which is prominent and are seen within 6 weeks after the transmission of the organism. The chancre is hard in appearance, with no pain, no fever, no illness and the person looks very normal. Sometimes ulcerous, it is self limiting and heals leaving a scar. Secondary symptoms extends from 2 to 5 years, rashes can be seen throughout the body especially in the trunk, extremities of limbs etc. Enlargement of the glands, certain lymph nodes and mucous lesions are also seen. Other mild symptoms include headache, malaise, sore throat, low fever, loss of voice and loss of pubic hair. Rarely liver abscess, kidney disease, arthritis, and optic neuritis happen. In latent phase, there are no symptoms for some duration of time, with no possibility of transmission of disease. After the latent phase, the organism becomes activated (tertiary phase). It is noninfectious and extends from 3-15 years.

Gummatous syphilis (soft tumour) - Gumma is found in different parts of the body like skin, bone, liver, spine etc. and patients face complications like senility, loss of hair and deformation of abnormal posture etc. Neurosyphilis is a condition where the organism reaches the central nervous system during which various complications are observed which include paralysis, deafness, and sterility. Cardiovascular syphilis (after 10 to 30 years) leads to abnormal functioning of aorta and aneurysm. The tertiary symptoms are permanent. In congenital syphilis, termination of fetus takes place, defective baby is born with physical abnormalities like abnormal skin, bones, joints, teeth etc and mental abnormalities like dementia, deafness, blindness, paralysis and cardiac problems. In some cases the baby remains asymptomatic for certain period with the development of symptoms later. The mode of transmission is sexual (vaginal/oral/anal sex), from mother to baby (congenital), kissing/fondling of genital organs, blood products transfusion, but is not transmitted by fomites.

Diagnosis can be done by observing clinical symptoms like chancre, knowing the patient history, performing blood tests like non treponemal (VDRL and RPR) tests and treponemal tests like TPA (treponemal plasma agglutination) and FTA (fluorescent treponemal agglutination reaction). Direct microscopy and PCR also help in the identification of the disease. It can be prevented by abstinence from intimate physical contact, having safe sex, avoiding contaminated blood, educating the general public and screening the pregnant woman.

Treatment of syphilis involves use of antibiotics. In early stage, penicillin and penicillin derivatives are used through intramuscular route. Alternative drugs are doxycycline, tetracycline, fluoroquinolones etc. During the later stages intravenous administration of above drugs in high doses is helpful.

Study of Gonorrhea

Gonorrhea is a common sexually transmitted infection caused by the bacterium Neisseria gonorrhoeae (Family Neisseriaceae). Neisseria gonorrhoeae is a spherical shaped, gram negative, fastidious bacterium existing as single or diplococci. It is a non-motile bacterium found in the cytoplasm of neutrophil. Its virulence factors are pili (for attachment), capsule (anti-phagocytic) and a potent endotoxin.

Signs and Symptoms: acute burning sensation with pain, symptoms of infection with N. gonorrhoeae differ, depending on the site of infection. About 10% of the infected males and 80% of infected females are asymptomatic. Infection of the genitals results in a purulent (or pus -like) discharge from the genitals, which is foul-smelling. Symptoms may include inflammation, redness, swelling, and dysuria. N. gonorrhoeae can also cause conjunctivitis, pharyngitis, proctitis or urethritis, prostatitis and orchitis. Conjunctivitis is common in neonates (newborns). Silver nitrate or antibiotics are often applied to their eyes as a preventive measure against gonorrhoea. Neonatal gonorrheal conjunctivitis is contracted when the infant is exposed to N. Gonorrhoeae at their birth canal which leads to corneal scarring or perforation, resulting in blindness of the neonate. Infection of the genitals in females with N. gonorrhoeae results in pelvic inflammatory diseases if left untreated, which results in infertility. In chronic stages, it leads to the inflammation of all important organs.

Transmission: it is transmitted from person to person during sexual interactions, through blood transfusions, mother to newborn during birth and rarely by fomites like sharing of needles etc.

Diagnosis: it can be diagnosed by isolation of the bacterium from the infected individual on a selective media containing antibiotics, by staining of urethral discharges and/or blood (neutrophils) - bacteria can be observed under microscope, by serological testing i.e. detection of antibodies in the infected individual, by biochemical identification i.e. oxidase test (reddish purple color with tetramethyl phenylenediamine reagent).

Vaccine: there is no successful vaccine that confers immunity against gonorrhea, all individuals are susceptible for this infection, and after recovery the immunity is only for a short period.

Prevention: avoid sex with infected partners or use safer sex methods, avoid contaminated blood transfusions, and with the use of ophthalmic drops neonatorum ophthalmic gonorrhea can be avoided.

Treatment : Antibiotics like penicillin with probenecid is most effective. Use of ceftriaxone or other cephalosporins controls the resistant forms. Complete treatment can be achieved by proper use of antibiotics.

Study of HIV

HIV is an acquired immunodeficiency syndrome, where the immune system is completely impaired and hence susceptible to many infections which is fatal. This is pandemic (epidemic all over the world), and is caused by human immunodeficiency virus. It is identified in 1981, by CDCP (Centre for disease control and prevention). There is no cure/no vaccine/no drugs. Drugs may control the disease and prolong the life of patient, but doesn't prevent disease. HIV virus belongs to group VI SSRNA reverse transcriptase, family retroviridae and genus lentivirus and species HIV 1 and HIV 2, it is roughly spherical, enveloped, with the help of glycoprotein 120 it enters the target cells CD4 and TH cells. HIV enters into the CD4 cells and multiplies. Cytotoxic T cells destroys the CD4 cells, resulting in decreased count known as acute phase. HIV from one CD4 enters into the adjacent CD4 cells, and the entry of virus from CD4 cells directly to other CD4 cells is known as direct phase and the entry of virus into CD4 cells through the biological fluids known as indirect phase. CD4 count decreases gradually in a phase known as chronic phase leading to decreased immunity and hence becomes susceptible to many opportunistic infections and death.

HIV was classified into four stages -

- Stage 1 - occur in 8 weeks, individual may be asymptomatic in 40 to 50% of the patients, and in some cases symptoms like headache, flu like symptoms, throat inflammation, weakness, large lymph nodes, nausea and vomiting.

- Stage 2 - extends upto 10 years, asymptomatic or mild symptoms like swollen glands can be seen, HIV decreases in blood, HIV antibody can be detected, at the end of the stage, weight loss, GI problems, lymphadenopathy are seen. CD4 count is less than 500/µl.

- Stage 3 - immunity is deteriorated, reduction in CD4 cells is markable, hence many opportunistic infections and cancer can occur, severe diarrhoea, vomiting, pneumocystis pneumonia and esophageal candidiasis are also seen. CD4 cells count can be less than 350/µl.

- Stage 4 - aggravation of opportunistic infections takes place leading to tuberculosis and pneumonia, Kaposi sarcoma, attack of herpes and flu virus, fungal and parasitic infections are also observed. CD4 count can be less than 200/ µl. Patient suffers with fever, weight loss, sweat, looks lean, with various infections along with cancer leading to death.

Diagnosis: History of the patient is very much essential in this disease before making up final diagnosis. Blood tests like ELISA, RIA are suggested if antibodies are detected in blood. PCR is an advanced test which gives further confirmation. Tests like southern blot test and biological fluids from gum tissues also help for detection. Various modes of transmission include unsafe sex, blood transfusion, mother to baby and other biological fluids (semen/vaginal fluids/milk). There is no risk with fecal matter, sputum, sweat, tears, nasal secretions, saliva, urine and vomiting until and unless blood is present in these secretions.

Prevention: Prevention of the infection is done by sex abstinence from suspected partners, or by following safe sex, avoid contaminated blood transfusions, safe delivery or termination of pregnancy, preventive therapy for mother by using antiretroviral drugs, use of disposable needles, maintenance of hygiene and educating public. No vaccine is available for the disease

Treatment: Disease can be controlled by prolonging the life of the patient by antiretroviral drugs like nucleoside reverse transcriptase inhibitors (e.g zidovudine), non nucleoside reverse transcriptase inhibitors (e.g nevirapine), and protease inhibitors (e.g. ritonavir). These drugs help in controlling the virus multiplication and thereby reducing the morbidity.