Pharm.D-Pharmaceutical Micorbiology

(2.2T)

Course teacher: Dr.V.Parthasarathy-Professor, Department of Pharmacy

Unit:8. Diagnostic test

Enzyme -Linked Immunosorbent Assay (ELISA)

Principle:

1. Enzyme linked immunosorbent assay is commonly known as ELISA or EIA. The principle involved in ELISA is similar to that of Radio Immuno assay rather enzyme replacing the radioactive label.

2. In this assay an enzyme conjugated with an antibody reacts with a colourless substrate to generate a coloured reaction product and the substrate is known as chromogenic substrate.

3. The enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and β-galactosidase. The ELISA is more sensitive and it has the advantage of being safer and less expensive.

4. ELISA used for the quantitative measurement of either antigen or antibody, alternatively it can be used for the quantitative estimation of antigen or antibody using standard curve based on known concentration of antibody or antigen.

5. Based on the purpose the ELISA can be classified in to (a) Indirect ELISA (b) Sandwich ELISA (c) Competitive ELISA.

INDIRECT ELISA

1. The qualitative and quantitative estimation of antibody can be carried out using indirect ELISA. In this method serum or some other sample containing primary antibody (Ab1) will be added to an antigen-coated micro titre well and allowed to react with the antigen attached to the well.

2. The un reacted Ab_1 will be removed by washing. The antigen bound antibody is detected by adding an enzyme-conjugated secondary anti-iso-type antibody (Ab_2), which binds to the primary antibody.

3. Then the free Ab_2 will be removed by washing and a substrate for the enzyme is added to develop the colour. The enzyme-substrate reaction is quenched by using **2**M H_2SO_4.

4. The intensity of yellow colour was measured at 450nm using specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds. The intensity the coloured reaction product is corresponding to the concentration of antibody. It is a method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV)/ any other viral infections including COVID-19.

5. In this method, recombinant envelope (*Env*) and core proteins of HIV are adsorbed as solid-phase antigens to microtitre wells. The serum antibodies to these HIV proteins from the HIV infected patients will be detected. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection.

SANDWICH ELISA

1. This method is used for the qualitative or quantitative estimation of antigen. In this assay, the antibody rather than antigen is immobilized on a microtitre well.

2. A sample containing antigen is allowed to react with the immobilized antibody. The un reacted antigen removed by washing and then coated with a secondary enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen.

3. The free secondary is removed by washing and then treatment with substrate solution will produce a coloured reaction product. The enzyme-substrate reaction is quenched by using **2**M H_2SO_4 .The intensity of yellow colour is measured at 450nm using a spectrophotometric plate reader.

COMPETITIVE ELISA

1. Another variation for measuring amounts of antigen is competitive ELISA. In this technique, the antigen coated wells of microtitre plate incubated with a mixture of antibody and a sample containing antigen.

2. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well.

3. Washing will remove the unreacted reaction mixture. Addition of an enzyme-conjugated secondary antibody (Ab_2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA.

4. In the competitive assay, however, the higher concentration of antigen in the original sample, the lower the absorbance.

Applications of ELISA

a. The ELISA is extensively used in biochemistry, cell biology for the qualitative detection of proteins, antigens, antibodies, enzymes.
b. Screening of donated blood for the evidence of anti HIV antibodies.
c. The ELISA is employed to confirm the presence of HIV1 and HIV-2 infection by detecting anti-HIV antibodies in a human serum sample or HIV antigens.
d. The expression levels of various enzymes/proteins by animal, human and plant cells are analysed.
e. ELISA measures the hormone such as LH, TSH, T3 and T4 in blood for thyroid functions.
f. ELISA detects the presence of Human chorionic gonadotropin (**hCG**) is a hormone to confirm pregnancy.
g. ELISA is used to detect the food and other allergens.

Reference book: Immunology –5 edition
Goldsby,R.A., Kindt,T.J., Osborne,B.A., Kuby,J. W.H.Freeman and company. NewYork. pp148-151.

Polymerase Chain Reaction (PCR)

1. Polymerase chain reaction (PCR) is a technique for the *in vitro* amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of DNA. The enzyme DNA polymerase catalysis the reaction.
2. The PCR method was devised and named by Kary Mullis in 1983 and colleagues at the Cetus Corporation, although the principle has been described by Khorana and colleagues. Kary Mullis shared the Nobel Prize in chemistry with Michael Smith in 1993.
3. As the name implies, it is a chain reaction, a small fragment of the DNA section of interest needs to be identified which serves as the template for producing the primers that initiate the reaction. One DNA molecule is used to produce two copies, then four, then eight and so forth.
4. PCR is a major development in the analysis of DNA and RNA because it has both simplified existing technology and enable the rapid development of new techniques in immunology, molecular biology and drug discovery.
5. Polymerase chain reaction employs two primers, each complementary to opposite strands of the region of DNA, which have been denatured by heating.
6. In this reaction DNA polymerase carry out the synthesis of complementary strands of DNA in the 5' to 3' direction using a single stranded technique, but starting from a double stranded region. This is knowna primer extension reaction.
7. Various steps involved in PCR are:

a. **Denaturation**

The DNA template is heated to 94° C. This breaks the weak hydrogen bonds that hold DNA strands together in a helix, allowing the strands to separate creating single stranded DNA.

b. **Annealing**

The mixture is cooled to anywhere from 50-70° C. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.

c. **Extension**

The reaction is then heated to 72° C, which is the optimal temperature for DNA polymerase to react. DNA polymerase extends the primers, adding nucleotides onto the primer in a sequential manner, using the target DNA as a template.

With one cycle, a single segment of double-stranded DNA template is amplified into two separate pieces of double-stranded DNA. These two pieces are then available for amplification in the next cycle. As the cycles are repeated, more and more copies are generated and the number of copies of the template is increased exponentially.

Types of PCR

a) Real-time PCR
b) Quantitative real time PCR (Q-RT PCR)
c) Reverse Transcriptase PCR (RT-PCR)
d) Multiplex PCR
e) Nested PCR
f) Repetitive sequence-based PCR

Applications of PCR

1. PCR is used in analyzing clinical specimens for the presence of infectious agents such as HIV, hepatitis, malaria, anthrax, Covid-19 etc.

2. PCR can provide information on a patient's prognosis, and predict response or resistance to drug therapy.

3. Many cancers are characterized by small mutations in certain genes, and this is what PCR is employed to identify.

4. PCR is used in the analysis of mutations that occur in many genetic diseases (e.g. cystic fibrosis, sickle cell anaemia, phenylketonuria, muscular dystrophy).

5. PCR is also used in forensics laboratories and is especially useful because only a tiny amount of original DNA is required, for example, sufficient DNA can be obtained from a droplet of blood or a single hair.

6. PCR is an essential technique in cloning procedure which allows generation of large amounts of pure DNA from tiny amount of template strand and further study of a particular gene.

7. The Human Genome Project (HGP) for determining the sequence of the 3 billion base pairs in the human genome, relied heavily on PCR.

8. PCR has been used to identify and to explore relationships among species in the field of evolutionary biology.

9. In anthropology, it is also used to understand the ancient human migration patterns. In archaeology, it has been used to spot the ancient human race. PCR commonly used by Paleontologists to amplify DNA from extinct species or cryo-preserved fossils of millions years and thus can be further studied to elucidate on.

10. The multiplex endpoint PCR technology offers a number of potential advantages, results are available in a matter of hours rather than days, the extreme sensibility facilitates detection of even minutes the amounts of pathogen DNA in clinical samples.

11. While microbiological culture is likely to remain a gold standard for infection diagnosis, there is growing interest at the potential of PCR technology to provide early, time critical information based on detection and recognition of bacterial or fungal pathogen DNA. By this new modern method, the results are chosen in perquisite for giving adequate antibiotics treatment as early as possible in order to improve the standard of care.

Image of DNA ladder (Left) and DNA from PCR (Right-Arrow)

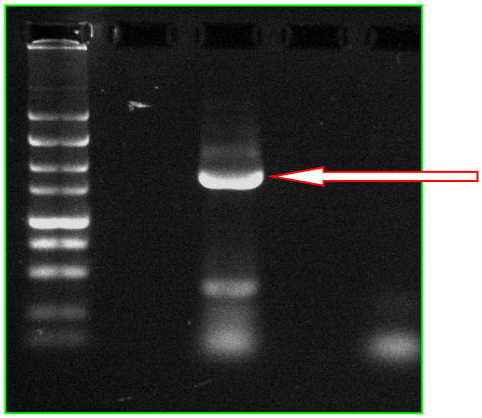


Image adopted from the thesis of Dr.V.Parthasarathy-Professor, Department of Pharmacy, AU.

Quantitative Buffy Coat (QBC) Test

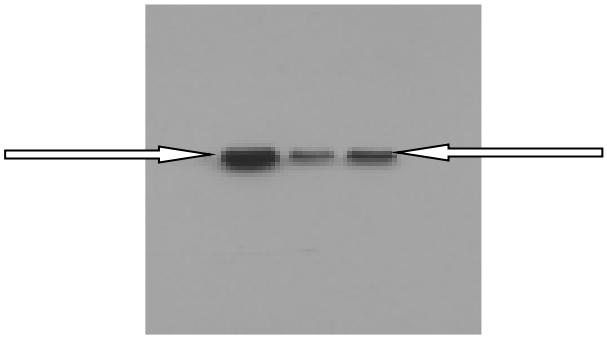
It is a new method for identifying the malarial parasite in the peripheral blood. This method was developed by Becton and Dickenson. This method involves staining of the centrifuged and compressed red cell layer with acridine orange, a dye. Then the cells are examined under UV light source. It is fast, easy and claimed to be more sensitive than the traditional thick smear examination.

Method:

1. To carry out the QBC test a QBC tube is used. The QBC tube is a high-precision glass hematocrit tube which is pre-coated internally with acridine orange stain and potassium oxalate.
2. The tube is filled with 55-65 microliters of blood from a finger, ear or heel puncture.
3. A clear plastic closure is then attached. A precisely made cylindrical float, designed to be suspended in the packed red blood cells, is inserted.
4. The tube is centrifuged at 12,000 rpm for 5 minutes.
5. The components of the buffy coat separate according to their densities, forming discrete bands. Because the float occupies 90% of the internal lumen of the tube, the leukocyte and the thrombocyte cell band widths and the top-most area of red cells are enlarged to 10 times normal.
6. The QBC tube is placed on the tube holder and examined using a standard white light microscope equipped with the UV microscope adapter, an epi-illuminated microscope objective. Fluorescing parasites are then observed at the red blood cell/white blood cell interface.
7. Since the parasites contain DNA which takes up the acridine orange stain, they appear as bright specks of light among the non-fluorescing red cells. Virtually all of the parasites found in the 60 microliter of blood can be visualized by rotating the tube under the microscope.
8. A negative test can be reported within one minute and positive result within minutes.

Western Blotting

1. Western blotting is a sensitive immunological method for detecting electrophoretically separated proteins.
2. Western blotting is also known as immunoblotting or protein blotting.
3. It is a widely used analytical technique in molecular biology and immunogenetics to detect specific proteins in a sample of tissue homogenate, blood serum or extract.
4. The western blot method employs gel electrophoresis to separate test proteins by 3-D structure or denatured proteins by the length of the polypeptide,
5. Followed by an electrophoretic transfer onto a membrane and the images recorded in the X ray films in dark room.
6. The common membranes used for western blotting are Polyvinylidene fluoride or polyvinylidene difluoride (PVDF) is a highly non-reactive thermoplastic fluoropolymer produced by the polymerization of vinylidene difluoride.
7. The technique was developed independently by several groups in 1979 and was later termed as 'western blotting' because of its analogy to Southern and northern blotting.
8. The following X ray image shows the western blot of protein sample after transferred into the nitro cellulose paper from agarose gel by gel electrophoresis.
9. The left arrow indicates the Standard Protein and the right arrow indicates test protein from blood serum/tissue etc.



Western blot image adopted from the thesis of Dr.V.Parthasarathy, Professor, Department of Pharmacy, AU.

Applications of Western blot

a. The **western blot** is extensively used in biochemistry, cell biology for the qualitative detection of single proteins and protein-modifications (such as post-translational modifications).

b. The confirmatory HIV test employs a **western blot** to detect anti-HIV antibody in a human serum sample.

c. It is employed to characterize the types of cancer with patient is suffering so to give specific treatment.

d. Western blot technique is very essential tool in cell biology, recombinant technology to analyze the expression levels of various enzymes/proteins by animal, human and plant cells analyses.

Southern blot

The **Southern blot** is a technique used in molecular biology to detect a specific DNA sequence in DNA samples. In this technique a transfer of electrophoretically separated DNA fragments to a filter membrane and followed by fragment detection by probe hybridization technique.

The Southern Blotting was developed in 1975 by Edward Southern. It was used to detect the sequence of DNA fragments. The, two other methods such as Northern and Western blotting are used to identify RNA and protein respectively.

Methodology of Southern blotting

1. The DNA fragments produced with restriction enzymes. The various sizes of the DNA fragments separated by gel electrophoresis.
2. Alkaline treatment used to denature the doubled-stranded DNA into a single strand. Then the DNA transferred into a nitrocellulose or nylon sheet by placing a membrane over the gel.
3. The flow of buffer solution promote the transfer of DNA from the gel into the membrane.
4. Capillary flow and vacuum transfer are the most common methods used to transfer the DNA fragments.
5. In capillary flow method, the gel is placed above the level of buffer on a supporting block with a membrane placed on the top.
6. A stack of absorbent towels are then placed over the membrane and are used to absorb the buffer from beneath the gel, lifting the DNA fragments up onto the membrane.
7. In the vacuum transfer method, the membrane is placed beneath the gel. The membrane and gel are submerged in buffer. A vacuum is then used to create a flow, which will pull down the DNA fragments into the membrane.
8. UV radiation or heating used to ensure that the DNA fragments remain attached to the membrane permanently.
9. Single-stranded labeled probes are then used to bind to target sequences and incubated.
10. The non-complementary probes are washed from the membrane, ensuring that only the bound probes remain.

11. Then the probes can be detected by autoradiography to reveal the pattern of hybridization on an x-ray film.

Applications of Southern blotting

1. Gene rearrangements can be analyzed using western blotting. For an example, in immunology this method used to identify the clonal rearrangements of T cell receptor genes.
2. It is used for restriction fragment length polymorphism (RFLP) and variable number tandem repeat (VNTR) analysis. RFLP used for DNA sequences to map genomes, which will be used in forensic medicine and paternity tests.
3. The presence of specific fragments of DNA in a mixture of many other fragments.
4. Southern blotting is used in the diagnosis of disease caused by mutation. For an example sickle cell anaemia, where a single nucleotide polymorphism (A to T) in the beta-globin gene leading to abnormal haemoglobin.
5. **S**outhern blotting is useful in the diagnosis of Fragile X syndrome, where increased CGG/CCG repeat region within the fragile X mental retardation 1 (FMR1) gene. The individuals with 55-200 repeats have a FMR1 gene permutation, where as an individuals with >200 repeats have fragile X syndrome.

References:

- http://bioinfosu.okstate.edu/MG/MGW4/MG423.html
- https://www.genome.gov/glossary/index.cfm?id=459
- www.cambridge.org/S0029665196000912
- https://link.springer.com/protocol/10.1007%2F978-1-60761-947-5_19
- www.nationaldiagnostics.com/overview-northern-and-southern-blotting
- www.cpet.ufl.edu/.../Southern-Blot-Handout-2011.pdf
- https://ghr.nlm.nih.gov/condition/fragile-x-syndrome#diagnosis
- Sofocleous, C. *et al* (2009) 'Molecular diagnosis of fragile X syndrome', *Expert Review of Molecular Diagnostics*, 9(1), pp. 23–30. doi: 10.1586/14737159.9.1.23.
- Wattendorf, D.J. and Muenke, M. (2005) 'Diagnosis and management of fragile X syndrome', *American Family Physician*, 72(01), pp. 111–113.

Pharm.D-Pharmaceutical Micorbiology

(2.2T)

Course teacher: Dr.V.Parthasarathy-Professor, Department of Pharmacy

Unit:10 Infectious Diseases

Definitions

Health: It is defined as a condition when the individual is in complete accord with surroundings or **A state of complete physical, mental and social well-being.**

Pathology: It is define as the study of suffering.

Pathogens: A microorganism that causes disease.

Disease: It is the loss of ease (comfort) to the body (dis-ease). However, it must be borne in mind that in health there is wide range of normality" ex. In height, weight, blood and tissue chemical composition etc. Thus, health and disease are not absolute but are considered as relative states.

Illness: A term commonly confused with disease is illness. Illness is the state of being unwell or an abnormal process in which aspects of the social, physical, emotional, or intellectual condition and function of a person are diminished or impaired as compared with that person's previous condition.

Epidemics: Widespread outbreaks of disease

Pandemics: Epidemics that spread internationally.

Causes of diseases:

The disease caused by

(a) Pathogens include bacteria, viruses and fungi;

(b) Toxins of pathogens or animals by damaging the cells of the host.

(c) Environmental pollutants.

(d) Malnutrition

CLASSIFICATIONS OF COMMUNICABLE DISEASES ACCORDING TO NATURE OF THE PATHOGEN

Communicable diseases are classified into nine types according to the nature of the pathogen/causing agent. They are (**1**). Viral diseases; (**2**). Mycoplasmal diseases; (**3**). Rickettsial diseases; (**4**). Chlamydial diseases; (**5**). Bacterial diseases; (**6**). Fungal diseases; **(7)**. Spirochaetal diseases **(8)** Protozoan diseases; (**9**). Helminthic diseases; (**10**). Diseases Caused by Nematodes (Round Worms).

Structure of HIV-1 virus

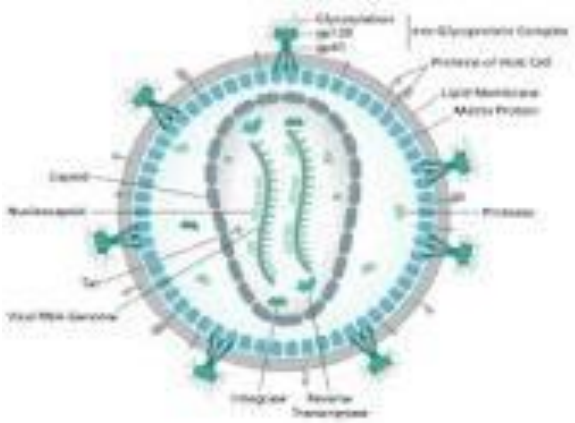


Image courtesy: www.google/Wikipedia.

Structural features of HIV

(i) The structure of HIV virus have single stranded RNA (ssRNA) genome and associated with it two molecules of reverse transcriptase enzyme (P64), which catalyse the reverse transcription of viral RNA into DNA in the host cells after infection.

(ii) The other proteins associated with the core of the virus include the P10 protease and P32 integrase.

(iii) Surrounding of the viral genome and the nucleoid proteins are two layers of core proteins p17 and p24.

(iv) The membrane modified by the insertion of two glycoproteins specific to HIV are gp120 and gp41.

(v) The gp120 is non covalently associated with gp 41.

(vi) The gp 41 spans across the membrane.

(vii) Both gp120 and gp41 help the virus to bind to cell membrane during the infection process.

The HIV envelop protein binds with $CD4^+$ and $CD8^+$ molecule of human T cells.

Modes of Transmission: Major route of transmission of HIV is through unprotected sexual intercourse, HIV infected blood transfusion, Intravenous (IV) drug abuse through shared needles, transplacental transfer from an infected mother to the foetus.

Incubation period: In majority of cases, HIV infected individual develop symptoms of AIDs between 8 to 10 years after an infection but in rare occasions 25% of the infected individuals have remained symptoms free for 10 to 12 years after infection.

Signs and Symptoms: The major symptoms are weight loss, chest infection (Tuberculosis, Pneumonia), fever, diarrohea, candidiasis, peripheral neuropathy.

Diagnosis: Study the serum profile of patient. Detect viral core protein P24 using Western blot or Radio Immuno Assay (RIA) or by Enzyme Linked Immuno Sorbant Assay (ELISA) using anti P24 antibody. Polymerase Chain Reaction (PCR) is a sensitive method used to identify the HIV glycoproteins.

Prevention and Treatment: No specific anti HIV drug is available until now. Hence, the combination of currently available antiviral drugs "Highly Active Antiretero Viral Therapy (HAART) is used to treat HIV infection. The viral drugs such as (a) Protease inhibitors (Saquinavir, Nelfinavir, Indianvir, Ritonavir); (b) Nucleoside reverse transcriptase inhibitors (Zidovidine, Abacavir, Lamivudine and Stavudine) are also used to treat HIV infection.

Viral Hepatitis

Symptoms: It is commonly called jaundice. Viral hepatitis is the most important form of hepatitis. In early stage the liver is enlarged and congested. In later stage the liver becomes smaller, yellowish or greenish. The symptoms in early phase include fever, anorexia, nausea, vomiting, epigastric discomfort, pains in muscles

and joints. The urine is dark and stool is pale. Splenic enlargement is sometimes present.

Types: There are 6 types of viral hepatitis. These are Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E and Hepatitis G. These (except Hepatitis G) are given below in table form. There is no Hepatitis F.

Characteristic Features of Different Types of Hepatitis:

Feature	**Hepatitis A**	**Hepatitis B**	**Hepatitis C**	**Hepatitis D**	**Hepatitis E**
Name of the virus	HAV	HBV	HCV	HDV	HEV
Nucleic Acid	RNA	DNA	RNA	RNA	RNA
Transmission	Faecal oral Route	Parenteral; (Blood, Needle, Body secretion, Placenta, Sexual contact)	Parenteral; (Blood)	Parenteral; (Blood, co-infection with hepatitis B)	Faecal oral Route
Symptoms	Fever, head ache, gastro intestinal disturbance, dark urine, jaundice	Similar, to HAV but no headache. Severe liver damage, yellowish eyes, light coloured stools,	Similar to HBV more likely to become chronic	Severe liver damage, high mortality rate	Similar to HAV but pregnant women may have high mortality
Incubation Period	2-6 weeks	6 weeks-6 months	2-22 weeks	6-26 weeks	2-6 weeks
Vaccine	Hepatitis A virus vaccine	Genetically modified vaccine	No	HBV vaccine is protective	No
Chronic Hepatitis	None	Yes	Yes	Yes	No

Typhoid (Enteric fever)

Pathogen: *Salmonella typhi*.

Modes of Transmission: Faecal oral route.

Typhoid Mary: It is a classic case in medicine. Mary Mallon was a cook by profession and was a typhoid carrier. She continued to spread typhoid for several years through the food she prepared.

Incubation Period: It is 1-3 weeks.

Signs and Symptoms: There is high fever but pulse rate is low. The patient feels abdominal pain and passes frequent stools. Confirmed by Widal Test. Typhoid vaccine is available.

Treatment: The patient is treated with antibiotics such as Terramycin and Chloromycetin.

Cholera

Pathogen: *Vibrio cholera*.

Modes of Transmission: Faecal Oral Route. Robert Koch (1843-1910) discovered cholera. John Snow (1913) was the first to demonstrate that cholera is transmitted by contaminated water.

Incubation period: It varies from a few hours to 2-3 days.

Signs and Symptoms: The patient starts passing stools frequently, which are white like rice water, and gets repeated vomiting. The disease can be diagnosed by the microscopic examination of the stool or the vomit when the typical comma-shaped cholera vibrio's can be seen.

Treatment: Rapid replacement of fluid and electrolytes is needed by oral rehydration- therapy. You can make your own oral rehydration solution (ORS) at home by adding one teaspoon of sugar and a pinch of salt to one quarter of water. Drugs tetracycline and chloramphenicol are used.

Tuberculosis (TB) or Koch's Disease

Pathogen: *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium micoti and Mycobacterium canet.*

Modes of Transmission: The bacteria damage the tissues and release a toxin named tuberculin which produces the disease. It affects the lungs, lymph nodes, bones and joints.

Modes of infection includes infection by inhalation of droplets expelled by tubercular patients, infection of food and drink contaminated with bacteria of tuberculosis, milk from a tubercular cow, etc.

Incubation period: 3-6 weeks (variable).

Signs and Symptoms: Symptoms of pulmonary (lungs) tuberculosis are fever, cough, blood containing sputum, pain in the chest and loss of weight, excessive fatigue, failure of appetite, slight rise of temperature in the evening, hoarseness of throat, night sweating and rapid pulse. Diagnosis of TB is done by Mantoux Test.

Prevention and Treatment: BCG vaccine gives protection against tuberculosis. When coughing, he/she should keep the handkerchief before his/her mouth. Tuberculosis is curable.

Isoniazid, Streptomycin and Rifampicin drugs are used to treat Tuberculosis.

Malaria

Pathogen: Malarial parasite (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae*). Plasmodium has two hosts:

(a) Female Anopheles Mosquito:

As the sexual phase of the malarial parasite occurs in the mosquito it is considered the definitive.

(b) Human beings: As the asexual phase of the malarial parasite occurs in man, it is considered the intermediate host. As the female Anopheles mosquitoes feed on

blood, only they can serve as vector hosts (= carrier) of malarial parasites. The parasite does not harm the mosquito.

Historical Aspects:

Lancisi (1717) first suspected a relationship between swamp, malaria and mosquito. Laveran (1880) discovered that malaria is caused by protozoan parasite. In fact he discovered Plasmodium. He got Nobel Prize in 1907. His topic of discovery was "Role of Protozoans in Causing Disease".

Golgi (1885) confirmed Laveran's discovery by observing stages of Plasmodium malariae in human RBCs. In 1897 Sir Ronald Ross, a doctor who was born at Almora in India and he was in Indian Army, established that malarial parasite is transmitted by the bite of a female Anopheles mosquito. In 1902, he got Nobel Prize for this discovery. He worked in India.

Life Cycle of Plasmodium:

Life cycle of Plasmodium requires two hosts for completion, such a two host life cycle is called digenetic.

I. Life Cycle of Plasmodium in Man:

1. Infective stage of Plasmodium is sporozoite. When the mosquito bites another human, sporozoites are injected with bite.

2. Parasites (sporozoites) reach the liver through blood.

3. The parasite reproduces asexually in liver cells, bursting the cell and releasing into the blood.

4. Parasites enter the red blood cells and reproduce asexually there bursting the red blood cells and causing cycles of fever and other symptoms. Released parasites infect new red blood cells.

5. Sexual stages (gametocytes) develop in red blood cells.

II. Life Cycle of Plasmodium in Female Anopheles mosquito:

(i). Female mosquito takes up gametocytes with blood meal.

(ii). Fertilisation and development take place in the mosquito's stomach.

(iii). The zygote elongates and becomes motile called ookinete.

(iv). The ookinete moves and bores through the wall of the stomach of female Anopheles mosquito. The ookinete changes to oocyst on the surface of the stomach.

(v). Inside the oocyst, sporozoites are formed which are released in the body cavity of the mosquito.

(vi). Mature infective stages (sporozoites) move to different organs of the body cavity but many of them penetrate salivary glands of the mosquito.

(vii). When the female Anopheles mosquito bites a healthy person, the sporozoites are injected in his/her blood alongwith saliva.

Human Species of Plasmodium and Types of Malaria:

In human beings, malaria is caused by four species.

(i). ***Plasmodium vivax:***

It is most common in India. It is less common in Africa. Its incubation period is about 14 days. It causes Benign Tertian Malaria. Recurrence of fever is after every 48 hours (every third day). Recurrent attacks of fever are called paroxysms.

(ii). ***Plasmodium falciparum:***

It is common in certain parts of India. It is the greatest killer of human beings over most parts of Africa and elsewhere in tropics. Its incubation period is about 12 days. Recurrence of fever is after every 48 hours (every third day). It causes Malignant (=Aestivo-autumnal or Pernicious or Cerebral or Tropical) Tertian Malaria.

(**iii).** ***Plasmodium malariae:***

It is common in tropical Africa, Burma, Sri Lanka and parts of India. It is less common in India. This was the species of malarial parasite discovered by Laveran. This is the only species which can also infect other primates. Its incubation period

is 28 days. Recurrence of fever is after 72 hours (every 4th day). It causes Quartan Malaria.

(iv). *Plasmodium ovale:*

This is the rarest of the four species which infect man. It is mostly found in tropical Africa. It is usually not seen in India. Its incubation period is about 14 days. It causes Mild Tertian Malaria.

Symptoms of Malaria:

The patient displays symptoms of malaria fever after a period of 14 days from infectious bite. Early restlessness, less appetite and slight sleeplessness are followed by muscular pains, headache and a feeling of chilliness. In response to chills the body temperature starts rising and may reach 106°F at the height of fever. The patient sweats a lot and the temperature steadily goes down to normal, till the next attack takes place after 48 hours.

Control of Malaria:

Malaria is widely spread disease in India. There is separate antimalaria department of the government which controls malaria through National Malaria Eradication Programme (NMEP).

(a) Treatment of malaria patient:

Quinine, the oldest drug for malaria, and other drugs are also used for this purpose. Quinine is extracted from the bark of the cinchona tree which is mostly growing in West Indies, India, Sri Lanka, Java and Peru. Other anti-malarial drugs are paludrine and Primaquin, Chloroquinine, Camoquin and Comoprima. Now malaria is also being treated with sulpha drugs such as sulphadoxin, dapsone, etc.

(b) Prevention of Infection:

Ducks, larvivorous fish like Gambusia, some adult insects like dragon flies, insectivorous plants such as Utricularia, are the natural enemies of mosquito larvae and pupae as they feed upon them. These may be introduced in the water containing the larvae and pupae.

Meningitis

Meningitis is inflammatory disorders of the meninges, which are the three membranes that cover the brain and spinal cord. Meningitis occurs when the meninges surrounded by the fluid and becomes infected. Mostly meningitis caused by viral, bacterial infections, fungal and parasites.

Meningitis caused by direct invading of bacteria through an ear, sinus infection, a skull fracture, or it may be due to surgeries. Several strains of bacteria can cause acute bacterial meningitis, most commonly caused by *Streptococcus pneumoniae* also known as pneumococcus.

In addition to these, Meningococcus also known as Neisseria meningitidis is the most commonest agent cause meningitis adolescents as well as middle aged individuals. But the Streptococcus pneumonia is again the most common causative bacterial organism causing meningitis in the elderly. Mycobacteriumare also a causative of meningitis. Old age and infant are prone to meningitis.

The meningitis also caused by virus. The Enteroviruses account for more than 85% of all cases of viral meningitis. These virus are belongs to the viral family Picornaviridae and include echoviruses, coxsackieviruses A and B, polioviruses, rhinoviruses, which cause the common cold.

Signs and symptoms in adult

The early symptoms of meningitis are similar to flu caused by influenza and the symptoms may develop after several hours or few days. The symptoms of meningitis are: High fever, severe headache, Stiff neck, nausea or vomiting, seizures, confusion, difficulty in walking, difficulty in concentration, loss of appetite or thirst.

Diagnosis

Meningitis diagnosed by culture test and CT scan.

Signs and symptoms in newborns

High fever, excessive sleepiness, poor feeding, continuous crying and stiffness of neck.

Treatment

Meningitis is treated with antibacterial and antiviral agent.